ANTIOXIDANT PROPERTIES OF SELECTED CULINARY FLOWERS

Kuruppu Appuhamillage Upeka Darshani **Weerasinghe**
& Katugampalage Don Prasanna Priyantha **Gunathilake***
Department of Food Science & Technology, Faculty of Livestock, Fisheries, & Nutrition, Wayamba University of Sri Lanka, Makandura, Gonawila, Sri Lanka
E-mail: *kdppgunathilake@yahoo.com

Abstract
Although the antioxidant potential of many plant parts including leaves, fruits and bark have been previously investigated, the that of flowers has remained speculative. Therefore this study was conducted to analyze the antioxidant properties of ten selected underutilized culinary flowers in Sri Lanka; Aponogetoncrispus, Lasiaspinosa, Sesbania grandiflora, Cassia auriculata, Aeglemarmelos, Hibiscus rosa-sinensis, Allium cepa, Brassica oleracea, Gmelina asiatica and Azadirachtaindica. Bioactive constituents; phenolics, flavonoids, anthocyanin, β-carotene, lycopene and vitamin C and antioxidant activities; antioxidant capacity, radical scavenging activity and reducing power of methanolic extracts of flowers were determined. The total phenolic content was in between 5.28±0.25 to 24.30±0.31 g GAE/kg FW with the highest in Cassia auriculata. The flavonoid and anthocyanin contents were ranged between 1.09±0.21 to 5.80±0.35g and of Rutin Equivalent (RE)/ kg of FM and 9±0.01 to 795±0.03 µg/100g DW respectively. β-carotene and lycopene contents were within 56.9±0.13-1070.4±0.26 and 18 ± 0.02 to 359±0.11 µg/g of DW respectively. Hibiscus rosa-sinensis, Cassia auriculata and Gmelin aasiatica showed the highest anthocyanin, β-carotene and lycopene contents respectively. The highest total antioxidant capacity was showed by Cassia auriculata. Azadirachta indica showed the highest DPPH radical scavenging activity and the reducing power among the studied flowers. Sesbania grandiflora showed the highest inhibition in hydroxyl radical scavenging activity. Vitamin C content was ranged between 0.35-174.1 µg/g of FM. In conclusion, this study revealed that the studied edible flowers are a good source of antioxidants and these flower species can be introduced as a promising source of natural antioxidants.

Keywords: anthocyanin; antioxidant activity; culinary flowers; polyphenolic; reducing power

Received: 25.04.2020 Reviewed: 10.07.2020 Accepted: 21.07.2020

1. INTRODUCTION

According to the WHO, the burden of chronic and degenerative diseases like cancers, cardiovascular diseases is rapidly increasing worldwide. In the development of these diseases, oxidative stress plays a major role (Pham-Huy*et al.*, 2008). Oxidative stress is an imbalance between free radical generation and elimination. The human body has several mechanisms to counteract oxidative stress by producing antioxidants (Pham-Huy*et al.*, 2008) and these antioxidants can be either endogenous or exogenous (Hajhashemi *et al.*, 2010). Endogenous antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) enzymes (MacNee*et al.*, 2001) and non-enzymic lipoid acid, glutathione, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins, transferrin, etc. (Valko *et al.*, 2007). Exogenous antioxidants are vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, zinc), phenolics, flavonoids, etc. and the body relies on dietary antioxidants to full fill the need (Hajhashemi *et al.*, 2010).

It is believed that an increased intake of food rich in natural antioxidants is associated with lower risks of degenerative diseases, particularly cardiovascular diseases and cancer (Diem *et al.*, 2013). Plants are the potential source of natural antioxidants and the number of antioxidant compounds synthesized by plants as secondary products, mainly phenolics, serving in plant defense mechanisms to counteract ROS (Apolo & Farma, 2008). Plants having many pharmacologically active compounds like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, fixed oils, which

are stored in their specific parts of leaves, bark, flowers, seed, fruits, root, etc. (Linn, 2013). These natural antioxidants that occur in plants vary between plant species as well as the plant parts (Kucekova*et al.*, 2013).

Although antioxidants from other plant parts; leaves, fruits, bark from aromatic, spicy, medicinal, and other plants were studied to develop natural antioxidant formulations for the food, cosmetics, and other applications, the culinary flower remains speculative. Flower also one of an important plant part which contains a great variety of natural antioxidants such as flavonoids, anthocyanin, and many other phenolic compounds (Kaisoon*et al.*, 2012). From ancient times, flowers have been used for human consumption in various cultures, such as European, Asian, East Indian, Victorian English, and Middle Eastern. Sri Lanka also has many underutilized edible culinary flowers that have been used traditionally for cooking and other medicinal purposes (Kumarathunga, 2008). For example, edible flowers such as *Cassia auriculata* (Ranawara), *Aeglemarmelos* (Beli), *Gmelinaasiatica* (Demata), etc. used in ayurvedic medicine while *Aponogetoncrispus* (Kekatiya), *Lasiaspinosa* (Kohila), *Sesbaniagrandiflora* (Kathurumurunga) flowers used as cooked dishes. Although Sri Lanka has a collection of culinary flower species, very fewer researches have been done on those flowers. Since finding new and safe antioxidants from natural sources is of great interest for applications in natural antioxidants, functional foods, and nutraceuticals, analyzing these edible flowers will be very important. Therefore in this study, some selected Sri Lankan edible culinary flower species have been tested to analyze their antioxidant properties.

2. MATERIALS AND METHODS

Materials

In this study, ten different culinary flower species were used. Those were *Aponogetoncrispus*(Kekatiya), *Lasiaspinosa* (Kohila), *Sesbaniagrandiflora* (Kathurumurunga), *Cassia auriculata* (Ranawara), *Aeglemarmelos* (Beli), *Hibiscus rosa-sinensis* (Pokuruwadamal), *Allium cepa* (Onion), *Brassica oleracea var. botrytis* (Cauliflower), *Gmelinaasiatica* (Demata) and *Azadirachta indica*(Kohomba). Flowerswere collected from Makandura, Pannala, Gampaha and Anuradhapura areas in Sri Lanka.

Sample preparation

Ten samples of flowers were washed with clean water and drained excess water. After measuring the weight, samples were dried in an oven at 40℃ until getting a constant weight. Then the dried samples were ground using motor and pestle after measuring final weight. After that, the ground samples were used for the extraction.

Extraction

The phytochemical extraction was done according to Bouterfas*et al.*, (2014) with some modifications. One gram of sample was mixed with 20 mL of 80% (v/v) aqueous methanol in a conical flask which is covered with an aluminum foil and then sealed with a parafilm. The flasks were kept on an orbital shaker for two hours. The sample mixture was then filtered through Whatman 4 filter papers and the filtrate was stored in a freezer at -18℃ until used for further analysis.

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu method (Singleton, 1999) was used to determine the total phenolic content with some modifications. Gallic acid was chosen as the standard and the data were presented as milligram Gallic acid equivalents (GAE)/ g fresh matter.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen*et al.*, 1999). The total flavonoid content of flowers was expressed as mg of quercetin equivalents / 100g fresh mass.

Determination of Total Anthocyanin content

The total anthocyanin content (TAC) was determined by the pH-differential method

(Giusti&Wrolstad, 2001).

Determination β-carotene and Lycopene content

β-carotene and lycopene were determined according to the method of Nagata & Yamashita (1992).

Determination of total Ascorbic acid content

The 2, 6-dichlorophenolindophenol titrimetric method (Nielsen, 2009) was used to determine the total ascorbic acid content of flowers with some modifications.

Determination of Total Antioxidant Capacity (TAC)

The method of Prieto *et al.*, (1999) was used to determine the total antioxidant capacity. The total antioxidant capacity of flowers was expressed as ascorbic acid equivalents per g of fresh matter.

Reducing power assay

The reducing power was determined by the method of Oyaizu (1986). Ascorbic acid was used as a positive control.

DPPH radical scavenging assay

The free radical scavenging activity was measured using 2, 2-diphenyl- 1 –picryl-hydrazyl (DPPH) by the method of Blois *et al.*, (1958). Different concentrations of each flower extract were prepared and the inhibition was measured to find out the IC_{50} value.

Hydroxyl radical scavenging (HO) assay

The scavenging ability for hydroxyl radicals is measured by the method of Kunchandy & Rao (1990).

Statistical analysis

All results are presented as mean ± SD (standard deviation). Data were analyzed for significance difference using one way ANOVA. Mean separation was done using the Turkey multiple variance test to determine the statistical differences among each flower species at a significance level of 0.05. Correlation between the total phenolic content and antioxidant activities of edible flowers was examined using Pearson's coefficient. All statistical analyses were carried out using SPSS 16 software package.

3. RESULTS AND DISCUSSION

Different bioactive constituents and the antioxidant activities of the ten studied flowers are shown in the Tables 1, 2 and 3. Though numerous studies reporting bioactive constituents in plant organs like leaves, fruits, the present work is the most comprehensive analysis of bioactive constituents for Sri Lankan culinary flowers. For example, (Goshwami *et al.*, 2012) have done a screening of antioxidant activity of *Lasiaspinosa* leaves. But in this study, the bioactive constituents and the antioxidant activity of the *Lasiaspinosa* flower is reported for the first time. In this study total phenolic, flavonoid, anthocyanin, carotene, lycopene and vitamin C content of ten culinary flower species were analyzed. Table 1 shows the total phenolic, flavonoids and anthocyanin content present in studied culinary flowers. As shown in Table 1 the total phenolic content of the flowers was in between the range of 5.28 ± 0.25 to 24.30 ±0.31 mg of GAE/g of fresh material (FM) and *Cassia auriculata* showed the highest value. The flavonoid content was within the range of 1.09 ± 0.21 to 5.80 ± 0.35 mg of RE/g of FM with the highest from *Aeglemarmelos*. The anthocyanin content was in the range of 9 ± 0.01 to 795 ± 0.03 µg/100g of dry matter (DM) and *Hibiscus rosa-sinensis* hasshown the highest value.

Plant foods are rich sources of phenolics, which are molecules that can act as antioxidants to prevent heart disease, reduce inflammation, lower the incidence of cancers, and diabetes, as well as reduce rates of mutagenesis in human cells (Khoddami *et al.*, 2013). In the studied culinary flowers, the total phenolic content ranged from 5.28 ± 0.25 to 24.30 ± 0.31 mg of GAE/g of fresh material (FM) and *Cassia auriculata* showed the highest value. These values are significantly higher than some founds in common kind of fruits and vegetables, e.g., strawberry (3.63 ± 6.7 mg of GAE/g of FM), oriental plum (6.68 ± 8.0 mg of GAE/g of FM), mulberry (15.15 ± 5.7 mg of GAE/g of FM), green pepper (2.06 ± 5.4 mg of GAE/g of FM), Ceylon spinach (2.69 ± 3.1 mg

of GAE/g of FM), beetroot (2.57 ± 0.7 mg of GAE/g of FM) reported in (Lin & Tang, 2007). And the value got for TPC of the *Allium cepa* flower (12.57 ± 0.93 mg of GAE/g of FM) is also significantly higher than the TPC of the bulb (red onion- 3.10 ± 4.9 mg of GAE/g of FM, white onion- 2.16 ± 1.4 mg of GAE/g of FM) of that species (Lin & Tang, 2007). Although Zhang *et al.*, (2012) have reported the TPC (2.53 ± 0.25 to 5.28 ± 0.41 mg of GAE/g of FM) and TFC (1.23 ± 0.17 to 2.27 ± 0.20 mg of RE/g of FM) of some of the culinary flowers used in other countries, the values got for this study (TFC, 1.09 ± 0.21 to 5.80 ± 0.35 mg of RE/g of FM with highest in *Aeglemarmelos*) are significantly higher than those values. For further studies, the phenolics and flavonoids present in these flowers can be evaluated separately.

Since anthocyanin is the largest group of the water-soluble pigments in the plant kingdom and responsible for cyanic colors ranging from salmon pink through red and violet to dark blue of most flowers (Strack& Wray, 1994) evaluating anthocyanin content of edible flowers is important to discuss the antioxidant potential of flowers. Because flowers are very colorful and when consuming these flowers mostly the water used as a liquid. In this study, the anthocyanin content got as 9 ± 0.01 to 795 ± 0.03 µg/100g of DM. *Hibiscus rosa-sinensis* showed the highest value and it is obvious when comparing the color of that flower with other studied flowers.

Table 2 shows the β-carotene, lycopene and vitamin C content present in culinary flowers. As mentioned in Table 2, β-carotene content fell between 56.9 ± 0.13 to 1070.4 ± 0.26 µg/g of DM and the lycopene content ranged between 18 ± 0.02 to 359 ± 0.11 µg/g of DM. The highest β-carotene content was observed in *Cassia auriculata* while the highest lycopene was observed in *Gmelinaasiatica*. Vitamin C content was ranged between 1.45 ± 0.11 to 176.34 ± 3.73µg/g of FM with the highest from *Cassia auriculata.* The β-carotene and lycopene are the antioxidants that have the singlet oxygen quenching ability and it is very important when discussing the antioxidant potential of plant materials. For this study β-carotene and lycopene content fell between 56.9 ± 0.13 to 1070.4 ± 0.26 µg/g of DM and 18 ± 0.02 to 359 ± 0.11 µg/g of DM respectively. And the highest β-carotene content was observed in *Cassia auriculata* while the highest lycopene was observed in *Gmelinaasiatica*. When considering lycopene, it is highest in tomato (380 µg/g of DM) (Agarwal & Rao, 2000) and the highest value got from this study is lower than that value. However, it is a comparable value. The β-carotene content is also comparably higher than the some of the studied vegetables in previous studies e.g., cabbage (90.10µg/g of DM), cucumber (28.0µg/g of DM), bottle guard (14.0 µg/g of DM), okra (322.0 µg/g of DM) (Ahamad *et al.*, 2007).

Table 1: Total phenolic content (TPC), Total flavonoid content (TFC) and total anthocyanin content of the studied edible flowers

Flower species	TPC (mg GAE/g of FM)	TFC (mg RE/g of FM)	Totalanthocyanin (µg /100g DW)
Hibiscus rosa-sinensis (Pokuruwadamal)	8.46 ± 0.22^{g}	5.04 ± 0.09^{a}	795 ± 0.03^{a}
Azadirachta indica (Kohomba)	22.87 ± 1.03^{c}	4.03 ± 0.08^{b}	45 ± 0.004^{b}
Allium cepa (Onion)	12.57 ± 0.93^{e}	1.50 ± 0.23^{c}	21 ± 0.01^{d}
Aponogetoncrispus (Kekatiya)	19.66 ± 0.42^{d}	5.01 ± 0.16^{a}	18 ± 0.01^{g}
Gmelinaasiatica (Demata)	9.30 ± 0.52^{g}	1.72 ± 0.18^{c}	22 ± 0.004^{c}
Lasiaspinosa (Kohila)	5.28 ± 0.25^{h}	1.09 ± 0.21^{d}	12 ± 0.01^{i}
Cassia auriculata (Ranawara)	24.30 ± 0.31^{b}	3.87 ± 0.11^{b}	11 ± 0.01^{j}
Aeglemarmelos (Beli)	18.90± 1.47^{a}	5.80± 0.35^{e}	20 ± 0.00^{f}
Sesbaniagrandiflora (Kathurumurunga)	11.50 ± 0.18^{f}	1.59 ± 0.07^{c}	17 ± 0.003^{h}
Brassica oleracea var. botrytis (Cauliflower)	11.28 ± 0.50^{f}	1.69 ± 0.28^{c}	9 ± 0.001^{e}

Each value in the table is presented as mean ± SD (n=3). Means within columns superscripted by the same letter are not significantly different at $p<0.05$, as measured by the Turkeymultiple variation test.

Table 2: β-carotene, lycopene and vitamin C content of the studied edible flowers

Flower species	Beta carotene (µg/g of DM)	lycopene (µg/g of DM)	Vitamin C (µg/g of FM)
Hibiscus rosa-sinensis (Pokuruwadamal)	56.88± 2.93^{i}	34.35± 2.09^{b}	1.98 ± 0.07^{i}
Azadirachta indica (Kohomba)	378.56± 6.75^{g}	56.62± 3.28^{c}	125.27 ± 5.57^{b}
Allium cepa (Onion)	748.80± 2.48^{d}	320.11± 9.22^{f}	2.39 ± 0.04^{g}
Aponogetoncrispus (Kekatiya)	619.60± 6.67^{f}	298.66± 0.98^{e}	63.47 ± 6.88^{c}
Gmelinaasiatica (Demata)	930.32± 6.78^{b}	359.12± 2.28^{g}	1.45 ± 0.11^{j}
Lasiaspinosa (Kohila)	867.44± 4.16^{c}	319.86± 8.24^{f}	2.37 ± 0.07^{h}
Cassia auriculata (Ranawara)	1070.40± 3.01^{a}	82.96± 7.24^{d}	176.34 ± 3.73^{a}
Aeglemarmelos (Beli)	742.20± 6.97^{d}	330.92± 5.96^{f}	11.34 ± 0.61^{d}
Sesbaniagrandiflora (Kathurumurunga)	252.24 ± 4.18^{h}	18.01± 2.07^{a}	4.52 ± 0.01^{e}
Brassica oleracea var. botrytis (Cauliflower)	662.56± 5.56^{e}	354.41± 4.55^{d}	2.92 ± 0.15^{f}

Each value in the table is presented as mean ± SD (n=3). Means within a column superscripted by the same letter are not significantly different at $p<0.05$, as measured by the Turkeymultiple variation test

Table 3: Total antioxidant capacity, DPPH radicals and Hydroxyl radicals inhibition% and reducing power of the methanolic extracts of edible flowers

Flower species	Antioxidant capacity AAE mg/g DW	DPPH radicals inhibition %	Hydroxyl radicals inhibition %	Reducing power (AAE mg/g of DW)
Hibiscus rosa-sinensis	0.42± 0.07^{c}	83.21	61.81	254.70 ± 0.07^{i}
Azadirachta Indica	0.82± 0.05^{a}	83.92	75.19	153.27 ±5.57^{b}
Allium cepa	0.30± 0.30^{d}	83.42	74.12	122.89 ±0.04^{g}
Aponogetoncrispus	0.21± 0.06^{e}	91.22	82.41	91.97 ± 6.88^{c}
Gmelinaasiatica	0.15± 0.07^{f}	27.12	74.12	68.03± 0.11^{j}
Lasiaspinosa	0.61± 0.09^{b}	93.70	81.26	244.27 ±0.07^{h}
Cassia auriculata	0.36± 0.03^{d}	83.49	74.61	318.36 ±3.73^{a}
Aeglemarmelos	0.66± 0.07^{b}	87.89	63.40	55.94 ± 0.61^{d}
Sesbaniagrandiflora	0.2± 0.06^{e}	90.65	73.28	85.92 ± 0.01^{e}
Brassica oleracea var. botrytis	0.42± 0.07^{c}	37.56	58.72	212.13 ±0.15^{f}

Each value in the table is presented as mean ± SD (n=3). Means within a column superscripted by the same letter are not significantly different at $p<0.05$, as measured by the Turkeymultiple variation test.AAE=Ascorbic acid equivalent

Though these flowers contain this much antioxidant compounds the contents can be varied at the consumption with the cooking or extraction methods. Here the methanolic extraction has been done and in cooking normally the water used. Since the antioxidant content varies with the extraction solvent (Sultana *et al.*, 2009) the further studies on the effect of cooking methods for the antioxidant potential of these flowers also will be very important.

Table 3 shows the total antioxidant capacity, reducing power and percent inhibition of radicals inhibition of the methanolic extracts of studied culinary flowers. All the bioactive constituent contents determined in this study have shown that the antioxidant potential of these studied flowers. For further proving some of the *in vitro* antioxidant activity assays also have been done. The total antioxidant capacities of the studied flowers were in the range of 0.82 ± 0.04 to 0.15 ± 0.01 mg of ascorbic acid equivalent / g of dry weight. The highest antioxidant capacity has shown by *Cassia auriculata* flower. The results for this test have been summarized in Table 3. To evaluate the antioxidant activity of a plant material one assay is not enough because they have different mechanisms such as free radical-scavenging, hydrogen-donation, singlet oxygen quenching, metal ion chelation, and acting as a substrate for radicals such as superoxide and hydroxyl (Robards *et al.*, 1999). So the methanolic flower extracts were analyzed in

DPPH radical scavenging assay, total antioxidant assay, hydroxyl radical scavenging assay and reducing power assay.

The total antioxidant capacity was determined and it may be more useful than determining one specific antioxidant compound or one antioxidant activity. This 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 medium. The total antioxidant capacity of the studied flowers was in the range of 0.82 ± 0.04 to 0.15 ± 0.01 mg of ascorbic acid equivalent / g of dry weight. The highest antioxidant capacity has shown by *Cassia auriculata* flower. That may be due to the highest phenolic, β-carotene, and vitamin C content of *Cassia auriculata* flower. Although in this study a good correlation between phenolic and antioxidant properties has not reported in some previous studies it has been reported (Kucekova *et al.*, 2013).

All the studied flowers were evaluated for their radical scavenging activity by using DPPH. As illustrated in Table 3 except two species *Lasiaspinosa* (Kohila)and *Aeglemarmelo* sother species showed percentage inhibition above 83% at the ten times diluted methanolic extract. *Aponogetoncrispus* showed the significantly highest percentage inhibition of 93.95 ± 0.90 %.The DPPH assay method is based on the reduction of DPPH, a stable free radical. When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor (e.g., a free radical-scavenging antioxidant) and is reduced to the DPPH. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. When higher the inhibition the antioxidant activity also higher in terms of hydrogen donating capacity (Leaves, 2014). *Aponogetoncrispus* (Kekatiya) showed the significantly highest percentage inhibition 93.95 ± 0.90 % among studies flowers and except two species *Lasiaspinosa* (Kohila) and *Aeglemarmelos* (Beli), other species showed percentage inhibition above 83% at the ten times diluted methanolic extract. All together the studied flowers have good hydrogen donating capacity. As shown in Table 3 all flower species showed above 61% inhibition except Beli. And among those nine flower species except Hibiscus and cauliflower, other flowers showed inhibition above 70%. *Sesbaniagrandiflora* (Kathurumurunga) showed the highest percentage inhibition of 82.38 ± 1.26%.

The IC_{50} value for the DPPH radical scavenging activity was calculated by measuring the scavenging activity of different concentrations and the lowest value showed by the *Azadirachtaindica* and the value is 190 mg/L of methanolic extract as shown in Figure 1. *Cassia auriculata* showed the second-lowest IC_{50} value of 230 mg/L of the methanolic extract (Figure 1).

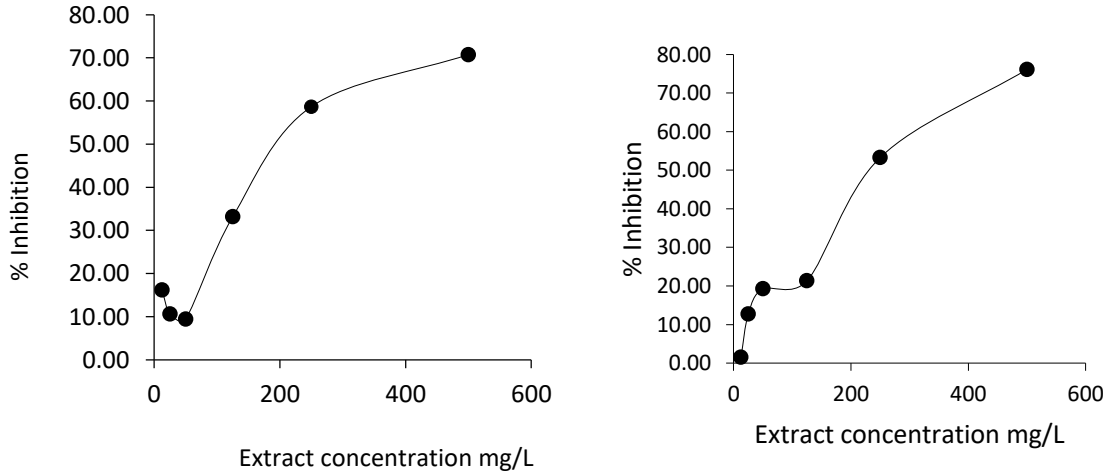

Figure 1: Percentage inhibition of flower extracts of Azadirachta indica (A) and Cassia auriculata (B) for DPPH radical scavenging activity with different concentrations.

For further evaluation, the IC_{50} value for these flowers was calculated. The IC_{50} value is the extract concentration need to inhibit 50% of the radicals. The lowest value showed by the *Azadirachtaindica* (Kohomba) and the value is 190 mg/L of methanolic extract and *Cassia auriculata* showed the second-lowest IC_{50} value of 230 mg/L of the methanolic extract. Though the *Aponogetoncrispus* showed the highest inhibition, the flowers of *Azadirachtaindica* resulted in the lowest IC_{50} value. Because the % inhibition is not changed linearly with the concentration of the extract and it gives a curve with different concentrations of the extracts.

Hydroxyl radicals are the major active oxygen species causing lipid oxidation and enormous biological damage. In hydroxyl radical scavenging assay ferric-EDTA was incubated with $H_2O_2$and ascorbic acid at pH 7.4 and hydroxyl radicals were formed in free solution and detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA at low pH form a pink chromogen (Gupta *et al.*, 2004). In this study, *Sesbaniagrandiflora* (Kathurumurunga) showed the highest percentage inhibition of 82.38 ± 1.26%. All flower species showed above 61% inhibition except *Aeglemarmelos*. And among those nine flower species except Hibiscus and cauliflower, other flowers showed inhibition above 70%. *Sesbaniagrandiflora* (Kathurumurunga) showed the highest percentage inhibition of 82.38 ± 1.26%. Polyphenols, tannins and flavonoids are very valuable plant constituents in the scavenging ability (Gupta *et al.*, 2004). For these results also the bioactive constituents of those flowers may be affecting.

Reducing power is associated with antioxidant activity and serves as a significant reflection of the antioxidant property of a commodity (Oktay*et al.*, 2003). Table 3 shows the reducing power of ten flowers is within the range of 68.03 ± 1.23 to 318.36 ± 2.46 mg of ascorbic acid equivalents per g of dry weight and the highest value shown by *Azadirachtaindica* (Kohomba) flower. Reducing power of flower extracts was evaluated by measuring the absorbance of 700 nm after mixing the extracts with ferric compounds. The presence of antioxidants in the extracts causes the reduction of the Fe3+/ferricyanide complex to the ferrous form. The samples with higher reducing power show higher absorbance. Reducing power of ten flowers is within the range of 68.03 ± 1.23 to 318.36 ± 2.46 mg of ascorbic acid equivalents per g of dry weight. The highest value is shown by *Azadirachtaindica* (Kohomba) flower. This reducing power is because they are electron donors. It was reported that the reducing power of polyphenolics is probably due to the presence of a hydroxyl group, which might act as electron donors (Shimada *et al.*, 1992).

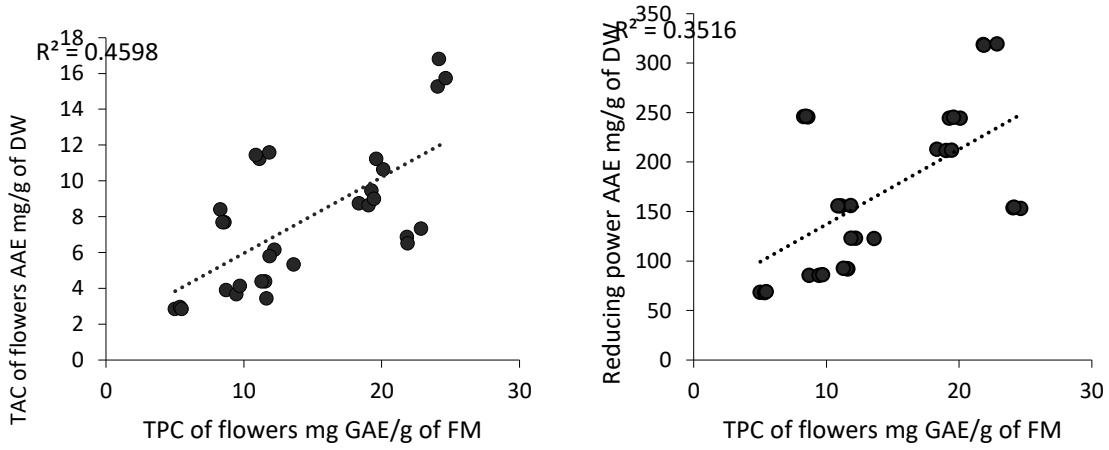

Figure 2: Correlation between the total phenolic content and the total antioxidant capacity (A) and between the total phenolic content and the reducing power

Therefore, they can act as primary and secondary antioxidants and can reduce the oxidized intermediates in disease-causing reactions.

In overall results, the correlation between the phenolic content and the antioxidant activities were not in a strong range. As illustrated in Figure 2 there is a weak positive relationship between the total phenolic content and the antioxidant activities; total antioxidant capacity and reducing power. However, according to previous researches, there is a strong relationship between the phenolic content and the antioxidant activity of flowers (Kaisoon *et al.*, 2012). There may be some errors during performing these assays cause of weak relationships. And also since here, the individual phenolic contents have not been analyzed the conclusions for the relationship change cannot be given. It will be useful if can analyze the phenolic compounds separately in these flowers because it has already shown that these flowers have higher phenolic contents than some of the fruits and vegetables.

4. CONCLUSION

Studied flowers have shown high content of bioactive constituents and remarkable variations in antioxidant activities. *Aeglemarmelos* showed the highest flavonoids content and *Hibiscus rosa-sinensis* showed the highest anthocyanin content. *Cassia auriculata* showed the highest phenolic, β-carotene and vitamin C content while the *Gmelina asiatica* showing the highest lycopene content. *Azadirachtaindica* showed the highest reducing power and the lowest IC_{50} value and *Cassia auriculata* showed the highest total antioxidant capacity. *Aponogetoncrispus* showed the highest DPPH radical scavenging assay and the *Sesbaniagrandiflora* and *Aponogetoncrispus* showed the highest hydroxyl radical scavenging activity. All these flowers can be introduced for the nutraceutical and functional food industries as a new promising source of antioxidants.

ACKNOWLEDGEMENT

We greatly acknowledge the support provided by the Wayamba University of Sri Lanka.

5. REFERENCES

[1]. Agarwal, S., & Rao, A. V. (2000). Tomato lycopene and its role in human health and chronic diseases. 163(6), 23–26.

[2]. Apolo, C. D. S., & Farma, F. De. (2008). Antioxidant activities of the extracts from chestnut flower, leaf, skins, and fruit. *Food Chemistry* 107, 1106–1113.

[3]. Blois, M.S. (1958) Antioxidant determination by the use of a stable free radical. *Nature* 181: 1199-1200.

[4]. Bouterfas, K., Mehdadi, Z., Benmansour, D., & Khaled, M. B. (2014). Optimization of Extraction Conditions of Some Phenolic Compounds from White Horehound (*Marrubium vulgare L.*) Leaves. *International Journal of Organic Chemistry* (4), 292–308.

[5]. Diem, Q., Elisa, A., & Tran-nguyen, P. L. (2013). Science Direct Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of Food and Drug Analysis*, 22(3), 296–302.

[6]. Goshwami, D., Rahman, M., Muhit, A., Islam, S., & Ansari, M. (2012). Antioxidant Property, Cytotoxicity and Antimicrobial Activity of Lasia spinosa Leaves. *Nepal Journal of Science & Technology*. 13(2), 215–218.

[7]. Guisti M.M, and Wrolstad R.E., 2003. Acylatedanthocyanins from edible sources and their applications in food systems. *Biochem Eng. J.* 14, 217-225.

[8]. Gupta, M., Mazumdar, U. K., & Gomathi, P. (2004). Antioxidant and Free Radical Scavenging Activities of Ervatamia coronaria Stapf. leaves, 119–126.

[9]. Hajhashemi, V., Vaseghi, G., Pourfarzam, M., & Abdollahi, A. (2010). Are antioxidants helpful for disease prevention? *Research in Pharmaceutical Sciences*, 5(1), 1–8.

[10]. Kaisoon, O., Konczak, I., & Siriamornpun, S. (2012). Potential health-enhancing properties of edible flowers from Thailand. *Food Research International*, 46(2), 563–571.

[11]. Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for Analysis of Plant Phenolic Compounds, 2328–2375.

[12]. Kucekova, Z., Mlcek, J., Humpolicek, P., & Rop, O. (2013). Edible flowers — antioxidant activity and impact on cell viability. *Central European Journal of Biology*, 8(10), 1023–1031.

[13]. Kunchandy E and Rao MNA. Oxygen radical scavenging activity of curcumin. *Intern. J. Pharmacognosy* (1990) 58: 237-240.
[14]. Leaves, L. (2014). Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides, 1(4), 244–249.
[15]. Lin, J., & Tang, C. (2007). Food Chemistry Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation, 101, 140–147.
[16]. MacNee W. (2001). Oxidative stress and lung inflammation in airways disease. *Eur. J. Pharmacol*.; 429:195–207.
[17]. Nagata, M., & Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. Nippon Shokuhin Kogyo Gakkaish, 39(10), 925–928.
[18]. Nielsen, S.S. (2009). Vitamin C Determination by Indophenol Method. In Nielsen, S.S. (eds) Food Analysis Laboratory Manual. USA: Springer US. p 55-60.
[19]. Oktay, M., Gülçin, I˙., &Küfreviog˘ lu, ÖI˙. (2003). Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. LWT - *Food Science and Technology*, 36(2), 263–271.
[20]. Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44, 307–315.
[21]. Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science* : IJBS, 4(2), 89–96.
[22]. Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269, 337– 341.
[23]. Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, 40(6), 945–948.
[24]. Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of FC reagent. *Methods in Enzymology*, 29, 152–178.
[25]. Strack, D., Wray, V. (1994). The Anthocyanins. In T*he Flavonoids: Advances in Research since* 9:1551–8.
[26]. Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts, 2167–2180.
[27]. Valko, M., Leibfritz, D., Moncola, J., Cronin, M.D. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Review. *Int. J. Biochem. Cell Biol*.; 39:44–84.
[28]. Zhang, Y., Yamamoto, S., Yamaguchi, T., & Kato, K. (2012). Application of paramagnetic NMR-validated molecular dynamics simulation to the analysis of a conformational ensemble of a branched Oligosaccharide. *Molecules*, 17(6), 6658–6671.