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Antioxidant potential of 12 medicinal plants of Sri Lanka

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Abstract
Antioxidants have the capability of decreasing adverse effects of reactive oxygen species. The present study was performed to evaluate the antioxidant properties of 12 medicinal plants which are used in the Aryurvedic medicinal system as well as reported to be used as dainty leafy vegetables in Sri Lanka. The qualitative screening of plants showed significant positive results of existence of all tested phytochemicals. The tannin concentration of all 12 plants tested was within the range from 0.41 - 29.51 mg TAE/g dw. Among the all tested plants, *O. octandra* showed remarkably high concentration of tannins (29.51 and 12.27 mg TAE/g dw), flavonoids (111.49 mg RE/g) and total phenolic contents (94.53 mg GAE/g dw). There was a significant correlation (P= 0.05) between total phenolics and three antioxidant capacities assays (FRAP, DPPH and ABTS). Similarly, there were high correlation between FRAP and ABTS assays (R^2= 0.912) and ABTS and DPPH assays (R^2= 0.873). Total chlorophyll content in twelve plants were ranged from 12.61 - 18.39 µg/ mL of fresh leaf samples. All plants evaluated showed significantly high amount of total chlorophyll content (>12 µg/ mL) including in *M. oppositifolia* which had exhibited less content of phytochemicals for all tests carried out in this study. The carotenoid content was ranged from of 0.68 - 1.59 µg/ mL. The results showed that all 12 medicinal plants had good antioxidant potential and they can be used in therapeutics as well as leafy vegetables for day today consumption.

Keywords: Medicinal plants, Total phenolic content, tannin, flavonoid, DPPH, ABTS, FRAP, chlorophyll, carotene.

Introduction
The history of plants in traditional medicine in Sri Lanka days back into 4th century before the Christian era. Since the Sri Lanka is rich in medicinal plants, nearly 1500 plant species including many endemics have been used Ayurveda, Siddha, Unani and Deshiya Chikitsa. The Ayurveda and Deshiya Chikitsa systems use mainly plant and herbal preparations for the treatment of diseases [1, 2]. Among them most of the plant extracts used in traditional medicine has been reported to have positive curing or controlling effects on the chronic diseases related to the oxidative stress and many previous investigations have revealed that many plant extracts does have antioxidant potential. So, the plant based approaches specially the edible plants for assessing antioxidant potential is vital for sustainable strategy to combat the alarming prevalence of oxidative stress related chronic diseases [3]. Antioxidants have the ability of decrease the harmful effects of reactive oxygen species (ROS) on regular physiological processes in human. Naturally many antioxidants such as catalase, superoxide dismutase (SOD) and glutathione peroxidase synthesized in the human body in order to provide the protection from the free radical damage. Nevertheless, human body produce more ROS (e.g., hydroxyl radicals, hydrogen peroxide and superoxide anion radicals) under stress condition more than enzymatic natural antioxidants. Therefore, the human body needs more supply of antioxidants to counteract with ROS [4]. Hence, constant consumption of synthetic or non-enzymatic antioxidants from the diet or as in the form of supplement is becoming essential.
However, synthetic antioxidant supplements such as butylated hydroxyl toluene (BHT), hydroxyl anisole (BHA), gallic acid esters and tertiary butylated hydroquinone have been prompt undesirable health effects and toxic which may cause liver damages and carcinogenesis In that sense the searching for non-enzymatic antioxidant constituents, which can be obtained from natural resources, have raised interest not only among scientists but also among food manufactures, producers and consumers for their role in the upkeep of human health [5]. Phytochemicals are groups of bioactive compounds consist of various natural antioxidants which include flavonoids, tannins, other phenolic compounds etc. and they have been

associated in the protection of human health against chronic degenerative diseases [6]. Phenolic compounds are popular group of secondary metabolites with wide pharmacological activities. Varied biological activities of phenolic acids were reported. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as staphylococcus aureus are some of biological activities of phenolic acids [7]. It has revealed that flavonoids and tannins have positive effects in reducing heart diseases and cancers respectively.

The present study was performed to determine *In vitro* antioxidant activities and their comparative characteristics in 12 medicinal plants. These plants are commonly found and used in the Ayurvedic medicinal system as well as reported to be used as dainty vegetables in Sri Lanka consumed as parts of the plant or whole plants (Table 1). A great number of analytical techniques are currently used for the quantitative and qualitative assessment of naturally available antioxidants in plant extracts[8]. These diverse protocols include determination of total phenol, tannin and flavonoids content, Folin-Ciocalteau, Ferric reducing antioxidant power assay (FRAP), 2,2-diphenyl-1-picrylhydrzyl (DPPH) and 2,2'-azino-bis(3-ethylbezothazoline-6-sulphonic acid (ABTS) radical scavenging activities.

The selected experimental methods were carried out to evaluate the total content of phenols, tannin and flavonoids in qualitatively and quantitatively along with the determination of total chlorophyll and carotene content. Selected antioxidant assays such as DPPH, ABTS, FRAP were applied to screen the 12 edible medicinal plants of Sri Lanka with the aim of using them as natural antioxidants in the form of 'nutraceutical formulations' in food and herbal medicine industries.

Materials and methods

Plant materials

Young fresh leaves of 12 plants were collected from three different locations (Bandarawela, Handapanagala and Ampara) of Sri Lanka (Table 1). All plants were taxonomically identified by Uva Provincial Department of Ayurveda, Ayurveda Commissioner's office, Diyathalawa, Badulla, Sri Lanka. The voucher specimens of the plant samples were deposited at the herbarium of the Department of Biological Science of South Eastern University of Sri Lanka.

Apparatus, reagents and chemicals

UV -Visible spectrophotometer (GENESYS 10S UV-Vis), Microcentrifuge (PLC-012E), Orbital shaker (88-881-102), Rotary evaporator (1001 VN/ R-3002). Ferric chloride, Lead (II) acetate -Anhydrous, Aluminum trichloride, Folin-Denis reagent, Rutin trihydrates, Sodium carbonate, Sodium hydroxide AR pellets, Sodium nitrite, Tannic acid, 2,2-diphenil-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Trichloroacetic acid, Folin-Ciocalteau reagent, Gallic acid monohydrate, Phosphate buffer (pH 7.4), Methanol, Potassium ferricyanide, L-Ascorbic acid.

Preparation of crude extracts

The young fresh leaves 12 plants were washed with distilled water and airdried under shade for 1-2 weeks at room temperature (30±2°C). The dried plant leaves were ground into fine powder by using domestic grinder and they were stored in air tight labeled dark lass vessels separately inside the refrigerator at 4°C for future use.

Table 1: Medicinal plants selected for the investigation.

Scientific Name	Common names	Medicinal uses	Parts of consumption
Impatiens repens (Moon)	Gal-demata (S) Kaasiththumbai (T) Ceylon Balsam (E)	Epilepsy, piles, insanity etc.	Leaves/ whole plant
Osbeckia octandra (L.) DC.	Heen Bowitya (S) Kathtoo mukhtohulai (T) Eight Stamen Osbeckia (E)	Anti-cancerous, diabetes mellitus, liver disorders etc.	Leaves and young stems
Argyreia populifolia (Choisy)	Girithilla (S) Sindu Kodi (T) Sri Lankan elephant creeper (E)	Heart diseases, diabetes mellitus, neurosis etc.	Leaves and shoots
Adenanthera pavonia L.	Madatiya (S) Aanai kundumani (T) Red bead tree (E)	Blood cleansing, chronic rheumatism, gout, bowel hemorrhages, Diarrhea etc.	Cooked seeds, young leaves
Abrus precatorius L.	Olinda (S) Kundumani (T) Indian liquorice (E)	Anti-bacterial, anti-fungal, anti-tumor, analgesic, anti-inflammatory etc.	Roots, Leaves (Seeds - Not recommended)
Leucus zeylanica (L.) R. Br.	Geta Thumba (S) Thumbai (T) Ceylon slitwort (E)	Anorexia, Skin diseases, flatulence, acute and chronic dyspepsia etc.	Leaves and young stem
Sesbania grandiflora (L.) Poiret	Kathurumurunga (S) Agati (T) Vegetable Hummingbird (E)	Aperient, diuretic, antibiotic, anthelmintic, antitumor and contraceptive properties etc.	Leaves and flowers
Moringa oleifera Lam.	Murunga (S) Murungai (T) Drumstick (E)	Anti - cancer, diabetes, anemia, arthritis etc.	Leaves, pods and flowers
Wattakaka volubilis (L. Fil.) Stap. f	Anguna (S) Kodi-palai (T) Green Milkweed Climber (E)	Emetic, diaphoretic, diuretic, Asthma, indigestion, leukoderma etc.	Leaves
Cardiospermum halicacabum L.	Wel-penela (S) Mutakkorran (T) Balloon vine (E)	Rheumatism, nervous diseases, rubefacient etc.	Leaves, stem and seeds
Mollugo oppositifolia L.	Hini-pala (S) Thura poondu (T) Green carpetweed (E)	Ant -diabetes, anti- malaria, joint pains, inflammation, intestinal parasites, furuncles etc.	Leaves and stem/ whole plant
Pergularia daemia (Forsssk.) Chiov.	Meda-hangu (S) Veliparuthi (T) Hariknot plant (E)	Anthelmintic, antibacterial, antifungal antiseptic, antivenin, emmenagogue, emetic expectorant etc.	Leaves

Qualitative screening of extracts for phytochemicals

A different but all possible various biochemical tests were carried out for all 12 crude extracts separately to check the presence/ absence of different phytochemicals as a preliminary analysis mainly targeting for phenols, tannins and flavonoids.

Test for phenols: A few drops of each crude extracts were mixed with 3-4 drops of 1% ferric chloride and the colour change of bluish black indicated the presence of phenols [9].

Test for tannins: A 3-5 drops of plant extract was diluted up to 10 mL by adding distilled water. Diluted sample was mixed well and boiled and then filtered through a filter paper (Whatman No. 40). 1% ferric chloride solution was added to the few drops of filtered extract and the presence of tannins was indicated by the development of brownish green or a blue-black colour change [10].

A 2 mL of diluted crude extract (50% v/v) was mixed with 1.6 mL lead acetate solution. Formation of white precipitate specified the occurrence of tannin [11].

Test for flavonoids: A few drops of crude extract was added to 2 mL of NaOH (2%). The availability of flavonoids in the crude extract was confirmed from the disappearance of intense yellow colour on the addition of few drops of diluted HCl. Similarly, a white precipitation confirmed the occurrence of flavonoids while adding 1 mL of lead acetate (10%) to 1 mL of crude extract [10].

Quantitative determination of total tannin, flavonoid and phenol

Total tannin content

The total tannin content was determined according to the method proposed previously [11]. A 0.5 mL of crude extract was reacted with 0.25 mL of Folin-Denis reagent diluted with distilled water (1:10) and the reaction mixture was further added with 0.5 mL saturated sodium carbonate. The total volume of the reaction mixture was made up to 5 mL by adding of 3.75 mL of distilled water. As soon as possible with in 30 min, the absorbance of the reaction mixture was measured at 700 nm. A similar reaction mixture without the crude extract was used as blank. The concentration of the total tannin was expressed with reference to tannic acid equivalent (mg TAE/g dry weight of leaves; Standard calibration graph: 0.01 – 0.05 mg tannic acid/ L, $Y = 3.36x + 0.038$, $R^2 = 0.96$).

Estimation of flavonoid content

A 2 mL of distilled water and 0.15 mL of aqueous sodium nitrite solution (NaNO2, 5% w/v) was mixed with 0.5 mL crude extract. A 0.15 mL aqueous aluminium trichloride solution ($AlCl_3$, 10% w/v) was added after allowing the reaction mixture to stand for 6 min. similarly, it was left for standing for another 6 min and then mixed with 2 mL of aqueous sodium hydroxide (NaOH, 4% w/v) solution. After making the total volume up to 5 mL by adding 2 mL of distilled water, the thoroughly mixed solution was allowed to stand for 15 min. The absorbance of the reaction mixture was measured at 510 nm. The blank was prepared in a similar manner by excluding sample. The concentration of total flavonoid content in the test samples was calculated from the calibration graph (0.01 – 0.05 mg Rutin/ L, $Y = 0.84x + 0.001$, $R^2 = 0.97$) and expressed as mg Rutin equivalent (mg RE/ g dry weight of leaves).

Estimation of total phenols by Folin-Ciocalteu

The available total phenols was determined using a phenol reagent 'Folin-Ciocalteau' with a slight modification of the original method [12]. Approximately 0.5 mL of crude extract was mixed with 0.5 mL of Folin-Ciocalteau reagent (0.5 M) and mixed and then incubated (at room temperature) for 30 min in the dark. It was further incubated in the dark for two hours after adding 3 mL of sodium carbonate (20%) and shaken appropriately. The absorbance was measured at 765nm using UV-Vis spectrometer. The total concentration of phenol was expressed as gallic acid equivalents (mg GAE/ g dry weight of leaves) with reference to the calibration graph constructed using gallic acid (0.01 – 0.05 mg GAE/ L, $Y = 6.47x + 0.01$, $R^2 = 0.99$).

Antioxidant capacity assays

Ferric reducing antioxidant power assay (FRAP)

The reducing power assay was performed according to the procedures recommended previously [13]. A 0.5mL of crude extract, 2.5 mL of phosphate buffer (pH 7.4) and 2.5 mL of aqueous potassium ferricyanide solution (1%, w/v) were mixed together and kept at kept at 50±2°C in water bath for 20min. After cooling, the mixture was centrifuged at 3000 x g for 10 min and 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL freshly prepared $FeCl_3$ solution (0.1%, w/v). The absorbance was measured 15 minutes after the initial mixing at 700nm and a similar mixture without the crude extract was used as blank. The results were stated in terms of L-ascorbic acid equivalent (mg/g dry weight of leaves) with reference to calibration graph (0.01 - 0.05 mg of ascorbic acid/ L, $Y = 3.39x + 0.07$, $R^2 = 0.98$).

DPPH radical scavenging activity

The ability of the plant to scavenge or inhibit the free radical DPPH was observed according to the protocol. A 100 µL of plant crude extract was mixed with 3.9 mL freshly prepared 0.1 mM methanolic solution of DPPH and the mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark [14]. At the end of the incubation period, the absorbance was measured at 517 nm with methanol as a blank. The % inhibition of the radicals was considered as equivalent to the antioxidant activity of leaf extracts and it was calculated using the following formula.

$$\% \text{ inhibition} = \{(Ab_{control} - Ab_{sample}) / Abcontrol\} \times 100$$

Where, $Ab_{control}$ was the absorbance of the DPPH solution without the addition of plant extract and Ab_{sample} was the absorbance of DPPH radical + plant extract.

ABTS radical scavenging activity

ABTS cation radical was produced by the reaction between equal amounts of ABTS (7 mM, 4 mL) and potassium persulfate (2.45 mM, 4 mL) solutions. It was stored in the dark at room temperature for 12-16 h before use. ABTS solution was then diluted with methanol to obtain an absorbance of 0.70 at 734 nm. ABTS radical scavenging assay was performed using a previously described procedure [12]. 5 µl of plant extract was added to the 4 mL of diluted ABTS solution. The absorbance was measured 30 minutes after the initial mixing at 734 nm. Methanol was used as a blank. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula.

% inhibition = $\{(Ab_{control} - Ab_{sample})/ Ab_{control}\} \times 100$

Where, $Ab_{control}$ was the absorbance of $ABTS^+$ and Ab_{sample} was the absorbance of $ABTS^+$ and plant extract.

Determination of total chlorophyll and carotene content

The chlorophyll and carotene contents were analyzed according to the previously described procedure [15]. Accurately weighted 0.5g of fresh leaf samples of six different plants were taken separately. They were grinded into a fine paste by using motor and pestle with 10 mL of 100% methanol. Homogenized sample mixtures were then centrifuge for 10,000 rpm for 15min. 5 mL of their supernatants were separated and they were mixed with 5 mL of 100% methanol (final volume 10 mL). The solution mixtures were analyzed for Chlorophyll-a, Chlorophyll-b and carotenoids content in UV-Vis spectrophotometer at 665.2 nm, 652.4 nm and 470 nm against methanol as blank. The concentrations (µg/ mL) of chlorophyll-a, chlorophyll-b and carotenoid were determined by using following equations:

Chlorophyll-a (C_a) = $16.72A_{665.2} - 9.16A_{652.4}$
Chlorophyll-b (C_b) = $34.09A_{652.4} - 15.28A_{665.2}$
Carotene = $(1000A_{470} - 1.63C_a - 104.96C_b)/221$

Statistical analysis

All experiments were carried out in three replicates and presented as mean± standard error (SE) using Minitab 17. All graphs were plotted using Microsoft Excel. One way analysis of variance (ANOVA) and statistically significant was considered at $P<0.05$. Pearson correlation test was applied to determine the correlation coefficient value (R^2).

Results and Discussion

Qualitative analysis of antioxidants

The screening of plants for bioactive compounds accommodate discovery of new medicinal drugs due to their role in cellular protection against the chronic disease [16]. The qualitative screening of plants showed significant positive results of existence of all tested phytochemicals such as tannin, phenols and flavonoids (Table 2). All kinds of testes used for the qualitative screening exhibited the high prevalence of different antioxidants in all most all plants. However, Wohler's reagent and ferric chloride exposed the absence of tannins in *Impatiens repens* and *Argyreia populifolia* respectively. In general, it is very difficult to correlate the intensity of colour change or the precipitation to the corresponding values of these phytochemicals that were tested quantitatively.

Quantitative analysis of antioxidants

Estimation of phenolic compounds: tannins, flavonoids and total phenols

The antioxidants activities of the plants can be evaluated using numerous methods. However, it is rather difficult to find a single method to estimate the total antioxidant activities because of the complexity of phytochemicals that prevails in the plant kingdom. Plant phenolic compounds are mainly classified into five major groups; phenolic acids, flavonoids, lignans, stilbenes and tannins depending on their structure [17]. The major plant phenolics such as flavonoids and phenolic acids are biosynthesized through the shikimic pathways. Although malonic acid pathway is also involved in the biosynthesis of some of these compounds (flavonoids); its role is rather minor in higher plants. Phenolic compounds possess one or more aromatic rings with one or more hydroxyl groups. Because of the electron donating capacity of hydroxyl groups phenols are able to scavenge reactive oxygen species it was identified that *In vitro* studies on antioxidants in which the higher antioxidant activity was demonstrated by phenolic compounds when compare to vitamins and carotenoids.

The tannin concentration of the methanolic extracts of all 12 plants tested was within the range from 0.41 - 29.51 mg TAE/g dw of the leaves (Table 3). It was revealed that *Osbeckia octandra* and *Impatiens repens* showed remarkably high concentration of tannins (29.51 and 12.27 mg TAE/g dw of leaves respectively). All the other plant extracts contained significantly very lower range of tannin content comparing with *Osbeckia octandra* and *Impatiens repens* leaves extract (0.41 - 1.90 mg TAE/g dw of the leaves).

Table 2: Qualitative screening of phytochemicals using different tests and reagents: Wohler's reagent, ferric chloride test, Alkaline reagent test and Lead acetate test.

Plant	Tannins		Flavonoids		Phenols
	Wohler's reagent	Ferric chloride	Alkaline Reagent	Lead acetate	Ferric chloride
Impatiens repens	+++	-	+++	+++	++
Osbeckia octandra	+++	+++	++	+++	+++
Argyreia populifolia	-	+++	++	+++	+++
Adenanthera pavonia	++	++	+++	+++	+++
Abrus precatorius	+++	+++	+++	+++	+++
Leucus zeylanica	+++	+++	+++	+++	+++
Sesbania grandiflora	++	+++	++	+++	++
Moringa oleifera	++	+++	+++	+++	++
Wattakaka volubilis	++	+++	+++	+++	++
C. *halicacabum*	++	+++	++	+++	+++
Mollugo oppositifolia	+++	++	+++	+++	+++
Pergularia daemia	+++	+++	+++	+++	++

(+++) - Very clear precipitate / color change
(++) - Clear precipitate / color change
(+) - Very little precipitate / color change
(-) - No color change

Tannins are abundantly present in many parts of the plant including leaves, flower buds, seeds, roots and stem. But they are mostly prevailing in the growth areas of trees, such as xylem and the secondary phloem and the layer between the cortex and epidermis. When we consider about the leaf tissues tannins are found most commonly in the upper epidermis and they evenly distributed specially in evergreen plants. Tannins are widely used for many medicinal uses due to their anti-inflammatory, anti-fungal, antioxidant and healing properties. Due to the anti-cancer property of tannins they are used for the prevention of cancer.

Flavonoids are important class of phenolic compounds which scavenge free radicals and metal chelates [18]. Since the flavonoids are directly involving prevention of arthrosclerosis, it has been proven that it reduces total cholesterol levels in blood and thereby lower risks of arthrosclerosis, teratogenicity and coronary disease. The antioxidant assays for quantifying the concentration of flavonoids in all tested plants had a range of 1.61 - 111.49 mg RE/g dw of leaves (Table 3). The highest content of flavonoids was in *Osbeckia octandra* (111.49 mg RE/g), followed by two species *Argyreia populifolia* and *Leucus zeylanica* which had almost equal amount of flavonoids (22.41 and 22.94 mg RE/g) respectively. All the other plant extracts contained low flavonoid content when comparing with *Osbeckia octandra* leaves and the lowest flavonoid content was observed in *Mollugo oppositifolia* (1.61 mg RE/g dw). The differences in the flavonoid structures and their substitutions influence the phenoxyl radical stability, thereby affecting the antioxidant properties of the flavonoids. It was confirmed that the major groups of flavonoids; Flavonones and flavanols which are mostly accumulated in epidermal layers of leaves and stem and they provide protection to the cell from UV-B radiation. Similarly, it has been recorded that the plants which are grown under tropical and high altitudes climate consist a large amount flavonoids due to continuous and overexposure to UV radiation [19]. Therefore, the present study also have confirmed the above statement since the *O. octandra* showed remarkable higher concentration of flavonoids. It is an endemic plant to Sri Lanka which belongs to the tropical region and also the young leaves of *O. octandra* were collected for the study from the mountain regions in Sri Lanka.

Table 3: Concentration of tannin and flavonoid of selected twelve plants (mg TAE/ g dw and mg RE/ g dw respectively).

Plant	Tannin	Flavonoid
Impatiens repens	12.27±0.11	14.23±1.18
Osbeckia octandra	29.51±3.68	111.49±4.70
Argyreia populifolia	0.62±0.03	22.41±0.74
Adenanthera pavonia	0.61±0.27	17.40±0.86
Abrus precatorius	0.49±0.03	16.89±1.78
Leucus zeylanica	1.90±0.05	22.94±0.55
Sesbania grandiflora	0.72±0.05	8.16±0.28
Moringa oleifera	0.44±0.11	15.94±4.89
Wattakaka volubilis	0.61±0.06	13.87±3.57
C. *halicacabum*	0.54±0.41	6.89±3.99
Mollugo oppositifolia	0.41±0.03	1.61±0.01
Pergularia daemia	0.97±0.17	6.10±0.01

The total phenolic content was expressed as mg GAE/g dry weight of fine powder with reference to the standard graph of gallic acid (y=6.47x + 0.01, R2=0.97). The total phenol content of methanol extracts of 12 plants was within a range of 0.64 - 94.53 mg RE/g dw of leaves (Table 4). Among the plants tested, *O. octandra* has shown the highest total phenolic contents (94.53 mg GAE/g dw) except all the other plants showed comparatively lower content of total phenol. *Adenanthera pavonia*, *Abrus precatoriu*, *Leucus zeylanica*, *Argyreia populifolia* exhibited slightly high antioxidant activities; 25.78, 22.92, 21.13 and 18.80 mg GAE/g dw respectively (Table 4).

However, among the plants studied *Mollugo oppositifolia* (2.29 mg GAE/g dw) and *Pergularia daemia* (0.64 mg GAE/g dw) contained significantly less amount of total phenolic substances. The evaluation of total phenolic content showed a remarkable variation in the plants used in this study. The present experiment exhibited the presence of phenolic substances in all 12 plants but with variable amounts. Among them, *Adenanthera pavonia*, *Abrus precatoriu*, and *Argyreia populifolia* probably can be used as rich source of natural antioxidants; bioactive polyphenols. Similarly, these results are accordance with previous findings which have proved the presence of high amount of phenolic compounds in green leafy vegetables including *O. octandra*, *L. zeylanica* and C. *halicacabum* [20], [33]. Phenolic compounds are universal important natural bioactive substances of vascular plants particularly in leafy vegetables, fruits and medicinal plants. It has been revealed that leafy vegetables, in general, have shown higher phenolic content as compared to fruit crops irrespective of cultivation systems [21], [26].

Table 4: Summarized results of total phenolic content, FRAP assay, DPPH assay and ABTS assay of selected twelve plants.

Plant	Total phenolic content (mg GAE/g dw)	FRAP assay (mg AAE/g dw)	DPPH assay (% inhibition)	ABTS assay (% inhibition)
Impatiens repens	8.43±1.04	1.85±0.07	89.18±1.55	91.76±12.66
Osbeckia octandra	94.53±8.30	8.49±0.56	87.57±0.29	97.29±0.29
Argyreia populifolia	18.81±1.49	2.52±0.08	58.53±2.55	99.29±0.25
Adenanthera pavonia	25.78±1.34	2.10±0.08	75.01±3.44	98.19±0.36
Abrus precatorius	22.92±1.34	1.60±0.11	83.81±2.53	97.05±0.08
Leucus zeylanica	21.13±2.13	2.88±0.07	63.99±2.70	98.00±0.62
Sesbania grandiflora	6.74±1.42	1.81±0.30	61.80±3.40	94.81±3.44
Moringa oleifera	6.93±1.90	2.10±0.24	75.60±2.90	99.10±0.22
Wattakaka volubilis	12.53±2.28	3.08±0.53	55.40±6.83	99.52±0.08
C. *halicacabum*	5.55±4.41	2.13±0.08	70.47±1.12	69.38±5.37
Mollugo oppositifolia	2.29±0.25	0.84±0.01	48.01±1.12	41.81±3.69
Pergularia daemia	0.64±0.24	1.49±0.09	52.05±9.24	69.31±9.19

Radical scavenging capacity

The antioxidant ability or the radical scavenging capacity of the plants were systematically determined using three main assays; FRAP, DPPH and ABTS and the results are shown in the Table 4.

These assays have been extensively used to determine the antioxidants activities or the capacity of plants as they involve with standard equipment to provide fast and reproducible results. The ferric ions (Fe^{+3}) reducing antioxidant assay power (FRAP) measures the ability of antioxidants which are present in the plant extract to serve as a reducing agent by donating a single electron to reduction of ferric ions (Fe^{+3}) to ferrous ions (Fe^{+2}) [27]. The ferric reducing antioxidant power was expressed as mg AAE/g dry weight of fine powder with reference to the standard graph of L-Ascorbic acid (y=3.39x + 0.067, R^2=0.976). The ferric reducing antioxidant power of the methanolic extracts of twelve plants were ranged from 0.84-8.49 mg AAE/g dw (Table 4 and Fig. 1a.).

DPPH (2,2-diphenyl-1-picrylhyrazyl; $C_{18}H_{12}N_5O_6$, M=394.33) has been characterized as a long-lived, organic, nitrogen stable free radical. On mixing the reaction mixture of $DPPH^{\cdot}$ and antioxidant contained extract produce color change from deep purple to yellow, similar color of hydrazine and finally produce a $DPPH_2$. Then the scavenging capacity of antioxidants towards $DPPH^{\cdot}$ can be evaluated by monitoring the decrease of its absorbance at 517 nm [29]. DPPH radical scavenging activity of all plant extracts were expressed according to the percent inhibition of DPPH radical. The percent inhibitions were varied from 48.01±1.12 - 89.17 ±1.55 % (Table 4). Among the plant extracts evaluated, *Impatiens repens*, *O. octandra and Abrus precatorius;* showed higher DPPH radical scavenging activity (89.17 ±1.55, 87.57 ±0.29 and 83.81 ±2.53) respectively compared with other leaf extracts evaluated. In addition. DPPH had a very positive strong correlation (R=0.951) with the total phenolic contents (Fig. 1b.). *Mollugo oppositifolia* and *Pergularia daemia* (48.01±1.12 and 52.05±9.24%) displayed significantly lower DPPH radical scavenging activity than all the other plant extracts.

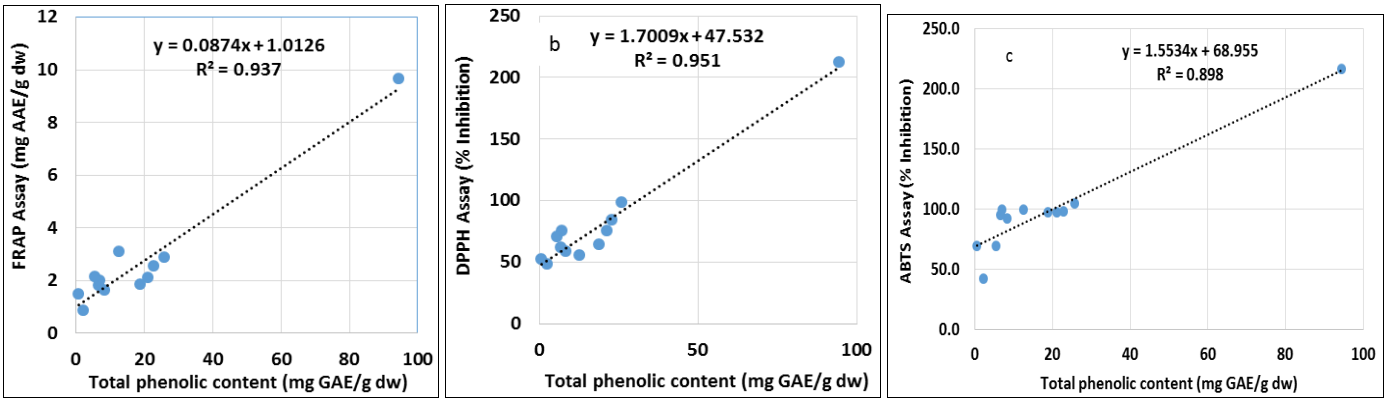

Fig 1: Correlation between total phenolics and three antioxidant capacities: FRAP assay (a), DPPH assay (b) and ABTS assay (c).

$ABTS^{\cdot-}$ was generated by persulfate oxidation of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$). $ABTS^{\cdot-}$ has a blue/green color with maximum absorption spectra at 734 nm, in methanol. With the presence of antioxidant it is decolorized from green [23, 30]. ABTS radical scavenging activity was expressed according to the percent inhibition of ABTS radical. The percent inhibitions were ranged from 41.81±3.69 - 99.29 ±0.25% (Table 4). Among all the plant extracts, *Impatiens repens*, *Osbeckia octandra*, *Argyreia populifolia*, *Adenenthera pavonia*, *Abrus pecatorious*, *Leucus zeylanica*, *Sesbania grandiflora*, *Moringa oleifera* and *Wattakaka volubilis* showed best ABTS radical scavenging assay. The ability of the plants to scavenge the radicals using phenolic compounds have been again proved with the strong correlation of ABTS assay (R= 0.898, Fig. 1c).

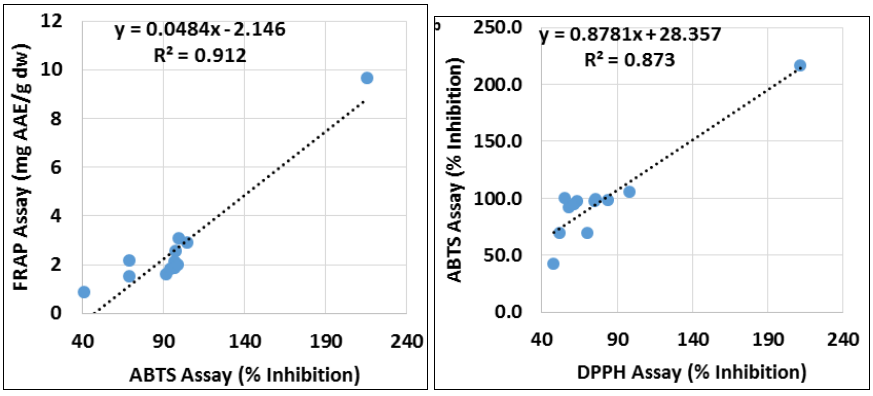

Fig 2: Correlation between FRAP and ABTS assays (correlation coefficient R^2= 0.912) and ABTS and DPPH assays (correlation coefficient R^2= 0.873).

A regression analysis was performed to correlate the different assays caried in this study. Significant correlations were found between the various methods used to determine the antioxidant potential especially between FRAP and ABTS assays (R=0.91, Fig. 2a) and DPPH and ABTS assay (R=0.87, Fig. 2b). The highest correlations were exhibited among FRAP, ABTS and DPPH assays, especially between FRAP and ABTS. Some studies about antioxidant potential of plant species demonstrated the direct relationship between their antioxidant activity and total phenolic content as phenolic compounds are one of the major constituents in plants and herbs [25], [31].

Estimation of chlorophyll and carotene

Chlorophyll possesses strong antioxidant capacity along with a significant amount of essential vitamins. These effective radical scavengers help neutralize harmful molecules caused as a consequence of oxidative stress [32]. Similarly, coloured vegetables and fruits are rich source of carotene which possess a number functional properties. Absorption from these foods is enhanced if eaten with fats, as carotenes are fat soluble, and if the food is cooked for a few minutes until the plant cell wall splits and the color is released into any liquid. 12 µg of dietary β-carotene supplies the equivalent of 1 µg of retinol, and 24 µg of α-carotene or β-cryptoxanthin provides the equivalent of 1 µg of retinol [33].

Total chlorophyll content in twelve plants were ranged from 12.61 - 18.39 µg/ mL fresh leaf samples. All plants evaluated showed significantly high amount of total chlorophyll content (>12 µg/ mL) including in *Mollugo oppositifolia* which has exhibited less content of antioxidants for all other tests carried out in this study. However, the higher amount of total chlorophyll can be observed in *Moringa oleifera* (19.26 µg/ mL) followed by *Abrus pecatorious* (18.39 µg/ mL), *Cardiospermum halicacabum* (18.14 µg/ mL) and *Argyreia populifolia* (18.10 µg/ mL) (Fig. 3). All the other plants also contained fairly good concentration of total chlorophyll. Carotenoid content was ranged from of 0.68 - 1.59 µg/ mL. Among the plant extracts analyzed *Mollugo oppositifolia* had the lowest carotenoid content (0.68 µg/ mL) and all the other plants contained significantly higher carotenoids (Fig. 3).

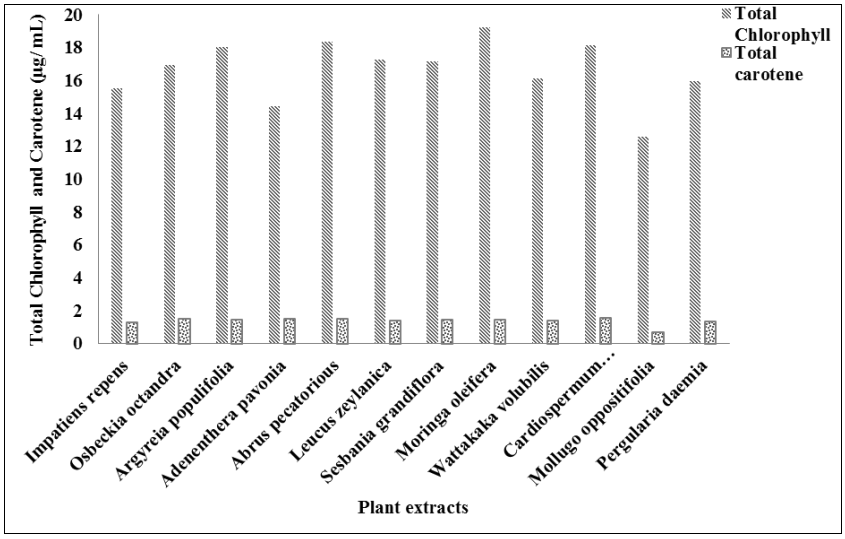

Fig 3: Quantification of total chlorophyll and carotene content (µg /mL) of twelve plants.

Carotenes are considered to be natural photo -oxidative protector in plants. Carotene and β-carotene can quench the singlet oxygen and capture peroxyl radicals and thereby showing the antioxidant properties [34]. In nature over six hundred carotenoids can be discovered. Among them about 40 are present in a usual human diet. Lutein is a dominantly prevailing type of carotene in leafy vegetables and many epidemiologic research have proven that high carotenoid intake will lead to decrease the prevalence of chronic diseases. But the biological mechanisms in carotenoids for such protection are presently unclear [34],[35].

Conclusions

The scientific data obtained from the screening of twelve plants have shown remarkable variations in antioxidant activities. It is obvious that they have good potential to be used in therapeutics as well as leafy vegetables for day today consumption. Among the twelve plants evaluated *Osbeckia octandra* revealed to be the most promising plant with the highest phytochemical content for good antioxidant activity. Highly significant correlation obtained for the ferric reducing antioxidant power assay as well as for the DPPH assay. The total phenolic content from the present study support that hypothesis that phenolic compounds contribute significantly to the total antioxidant capacity of the examined twelve plant species in Sri Lanka.

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