Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2017; 5(1): 91-95

Received: 14-11-2016
Accepted: 15-12-2016

Chethana Galketiya
Department of Pharmacy,
Faculty of Allied Health Sciences
University of Peradeniya,
Sri Lanka

T Sampath Weerarathna
Department of Pharmacy,
Faculty of Allied Health Sciences
University of Peradeniya,
Sri Lanka

J Chamini Punchihewa
Department of Pharmacy,
Faculty of Allied Health Sciences
University of Peradeniya,
Sri Lanka

M Nirmali Wickramaratne
Department of Physical Sciences
and Technology, Faculty of
Applied Sciences, Sabaragamuwa
University of Sri Lanka,
Belihuloya, Sri Lanka

DBM Wickramaratne
Department of Pharmacy,
Faculty of Allied Health Sciences
University of Peradeniya,
Sri Lanka

Correspondence
M Nirmali Wickramaratne
Department of Physical Sciences
and Technology, Faculty of
Applied Sciences, Sabaragamuwa
University of Sri Lanka,
Belihuloya, Sri Lanka

Screening of edible plants in Sri Lanka for antioxidant activity

Chethana Galketiya, T Sampath Weerarathna, J Chamini Punchihewa, M Nirmali Wickramaratne and DBM Wickramaratne

Abstract
Antioxidants play an important role in reducing oxidative damage to tissues, and therefore they are used in treating and preventing many diseases. Use of natural antioxidants in the form of edible greens in daily meals or as ayurvedic decoctions are encouraged due to its cost effectiveness and effectivity. The present study was carried out to determine antioxidant activities of 14 edible plants using 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, total phenolic content using Folin-Ciocalteu method and total reducing power using ferric chloride reducing method. The best DPPH scavenging activity was observed in *Costus speciosus* 4.63 ±0.13 µg/mL. *Murraya koenigii* exhibited the highest total phenolic content with 137.39 ±1.35 EGA (mg)/extracts (g), and showed the best total reducing capacity. These experimental results reveal that all edible plant extracts tested show anti-oxidant activity and those uncommonly used plants have comparatively better activity than the commonly used edible greens.

Keywords: Edible plants, DPPH Antioxidant activity, total reducing power, total phenolic content

1. Introduction
Use of plant extracts in traditional medications have been in the practice for long years and has exhibited a great potential in relieving the symptoms of many disease [1]. Plants make a major contribution to health care in especially in developing countries due to its cost effectiveness and the medicines derived from plant products are generally accepted to be safer than their synthetic counterparts and show fewer side effects [2, 3]. Antioxidants and plants have a close relationship and many plants are used as antioxidants in decoctions made in ayurvedic medicines. Consumption of edible plant as greens and vegetables has been in the practice for long years as a source of vitamins, fibers, antioxidants, and other medicinal values. Natural antioxidants in the form of raw extracts, decoctions or isolated chemical constituents are reported to be very effective to prevent the destructive processes caused by oxidative stress [4]. Therefor many studies are performed in obtaining scientific information on the antioxidant properties of medicinal plants and a large number of medicinal plants have been investigated.
Antioxidants play an important role in neutralizing the effect of reactive oxygen species (ROS) and other oxidants that lead to numerous diseases and complications. The human body is equipped with its own inherited antioxidant defense system that involves many enzymes as superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalases. These enzymes help to neutralize free radicals and reactive oxygen species and also to protect against free radical-induced cell damage. However, to accommodate excessive radical or reactive species produced due to many disease conditions it require the use of antioxidants in the form of supplement. Antioxidants are used as anti-mutagenic, anti-carcinogenic, and anti-aging agents [5, 6] since they can stabilize or deactivate free radicals generated as a result of the disease [7]. The role of free radical reactions in diseases such as diabetes, aging, immunosuppression and neurodegeneration pathology are well understood [8].
In the past few years the trend towards using naturally occurring antioxidants as food, cosmetic, and pharmaceutical products has increased as they possess enormous capability in performing its activity in a large magnitude and at various conditions with fewer side effects [9, 10]. Many naturally occurring compounds such as phenolic compounds, flavonoids, tannins ascorbic acid etc. contribute to the antioxidant properties of herbal plants, vegetables, and fruits.

The present study was performed on 14 plants which are used as green and vegetables and also has reported to be used in the Ayurvedic medical system in treating many diseases (Table 1) [11]. We investigate the antioxidant properties (TPC, DPPH scavenging activity, and the total reducing power) of these edible greens which are commonly used among the sub-urban population and three green vegetables namely kangkung, mukunuwenna and gotukola which are popular among the urban population.

2. Materials and Methods

All chemicals used were of analytical grade. Ferric chloride ($FeCl_3$), Folin-Ciocalteu; 2,2′-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), Sulphuric acid, sodium hydroxide (NaOH), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2) Potassium ferricyanide [K_3Fe (CN)$_6$]; trichloroacetic acid; sodium dihydrogen phosphate (NaH_2PO_4) and Methanol were purchased from Sigma. UV-visible spectrums were recorded on scanning UV-visible spectrophotometer (Thermo Fisher Scientific G10S).

2.1 Plant collection and extract preparation

The plants were collected from Gampaha and Kandy districts in Sri Lanka. The plant materials were botanically identified by the Natural Herbarium, Peradeniya Botanical Gardens, Peradeniya Sri Lanka. The fresh, plant materials (the edible part leaves, stems, root or up roots) were air dried and was coarsely powdered. A 500 g of each sample was extracted into methanol at room temperature for 2 x 24 hrs and the extracts were filtered and evaporated under reduce pressure using a rotary evaporator at 40 °C. The residues were dried under vacuum and was refrigerated until use.

2.2 Radical Scavenging Activity

The free radical scavenging activity of the extracts was measured using 2, 20- diphenyl-1-picrylhydrazyl (DPPH) assay. The stock solution of DPPH (1 mg/ ml) was prepared and was diluted with methanol to obtain an absorbance around 0.98 ± 0.02 at 517 nm. The extracts of concentrations ranging from 0.5 - 10 mg/ml were prepared. The extract (200 µl) was mixed with 3 ml of the diluted DPPH solution and was incubated in the dark, at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the positive control and the test was performed similar to the above procedure. The blank was prepared by replacing the extract with 200 µl of methanol and following the same procedure as stated above. The scavenging activity was estimated by calculating the percentage of DPPH radicals scavenged by the extract, using the equation given below [12]

$$\% \text{ DPPH Scavenging activity} = 100 \times \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}}$$

2.3 Total Phenolic Content

Folin-Ciocalteu's assay was used to determining the total phenolic content. The extract (1 mg/ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent and allowed to stand for 5 min. A 7% Na_2CO_3 (10 ml) w

as added to the reaction and was diluted with the addition of 13 ml of distilled water. The mixture was kept for 90 min, in the dark at room temperature, and absorbance was measured at 750 nm. The calibration graph was prepared using Gallic acid as the standard and the total phenolic content was expressed as milligrams of Gallic acid equivalents (EGA) in one gram of extract (EGA mg/ Extract g) [13].

2.4 Total Reducing Power

The potential of reducing Fe^{3+} to Fe^{2+} in the presence of the extract was measured spectrophotometrically at 700 nm. Each extract (500 µl) with varying concentrations was diluted with 1.25ml of phosphate buffer (pH 6.6) and was mixed with 1.25ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] and incubated at 50 °C for 30 min. A solution of 10% trichloroacetic acid (1.25 ml) was added to the mixture and was centrifuged at 3000 rpm for 10 minutes. A volume of 1.25 ml from the upper layer was pipetted out carefully and was mixed with 1.25 ml of distilled water and 250 µl of 0.1% ferric chloride ($FeCl_3$). The absorbance of the resulting solution was measured at 700 nm. The changes in the absorbance at 700 nm with the concentration, is an indication of the reducing power of the extract and rapid the changes in absorbance observed at low concentrations indicates better the reducing power of the extract [13].

3. Results

3.1 Free Radical Scavenging Activity

A solution of 2, 2,2'-diphenyl-1-picrylhydrazyl (DPPH) was used to measure the free radical scavenging activity of the extracts and the % of scavenging was calculated. Using the Plot of % scavenging vs concentration the IC_{50} values of each extract was calculated. The IC_{50} values of plant extracts of *Costus speciosus* < *Eleusine indica* < *Sida rhombifolia* Upper root< *Murraya koenigii* < *Ipomoea aquatic* < *Olax zeylanica* < Ipomoea batatas < *Aporosa lindleyana* < *Scoparia dulcis* < *Sida rhombifolia* root < *Centella asiatica* < *Alternanthera sessilis* < *Canthium coromandellicum* < *Coccinia grandis* < *Coriandrum sativum* were 4.63 ±0.13, 9.04 ±0.17, 11.0 ±0.17, 11.67 ±0.29, 22.20 ±0.45, 30.88 ±0.62, 51.19 ±0.29, 63.13 ±0.20, 109.06 ±0.21, 135.80 ±6.16, 155.00 ±2.77, 211.32 ±3.72, 255.59 ±1.47, 262.48±3.02, and 842.63 ±0.14 µg/mL respectively. The positive control Ascorbic acid showed an IC_{50} of 2.89 µg/mL.

3.2 Total Phenolic Content

The total phenolic content of the plant extracts were determined using Folin-Ciocalteu's assay and was expressed as milligrams of Gallic acid equivalents (EGA) in one gram of extract (EGA mg/ Extract g). TPC of the plant extract *Murraya koenigii* > *Alternanthera sessilis* > *Aporosa lindleyana* > *Coccinia grandis* > *Centella asiatica* > *Ipomoea batatas* > *Costus speciosus* > *Ipomoea aquatic* > *Canthium coromandellicum* > *Scoparia dulcis* > *Eleusine indica* > *Olax zeylanica* > *Sida rhombifolia* upper root > *Coriandrum sativum* > *Sida rhombifolia* root**,** with values of 137.39 ±1.35, 125.12±1.13, 99.46 ±1.86, 67.54 ±0.26, 55.00±0.56, 44.95 ±0.23, 38.71 ±1.94, 37.90 ±0.06, 22.99 ±0.56, 18.171 ±0.23, 14.50 ±0.69, 12.95 ±1.52, 10.98 ±0.17, 10.12 ±0.11 and 3.07 ±0.06 EGA (mg) / extracts(g) respectively.

3.3 Toral Reducing Power

The total reducing power of the extracts were measured using the potassium ferricyanide method which measures the potential of reducing Fe^{3+} to Fe^{2+} in the presence of the extract. The results were compared to the reducing potential of Gallic acid and the reducing potential of Gallic acid > *Murraya koenigii* > *Ipomoea aquatic* > *Olax zeylanica* > *Aporosa lindleyana*> *Coriandrum sativum*[2]> *Costus speciosus* > *Eleusine indica* upper root> *Alternanthera sessilis* > *Coccinia grandis* > *Sida rhombifolia* upper root > *Centella asiatica* > *Ipomoea batatas* > *Scoparia dulcis* > *Sida rhombifolia* root (not shown in graph) > *Canthium*

coromandellicum (not shown in graph). Therefore, the results indicate that *Murraya koenigii* extract to carry the best reducing potential of all plant extracts.

4. Discussion

Free radicals are associated with many diseases, causing side effects and complications. Antioxidants have the capacity to retard or inhibit the reactions initiated by reactive oxygen species and other radicals [14]. Therefore, use of antioxidants in controlling such conditions has been in the practice throughout. In this study, we determined the antioxidant potential of 14 plant extracts which are used as edible greens mostly among suburban population, using three different techniques as DPPH radical savenging activity, estimating the total phenolic content using Folin-Ciocalteu method and determining the total reducing power of the extracts.

The DPPH radical scavenging assay enables us to measure the electron donating ability of the extract and the scavenging of radicals are indicated by the extent of the decolourisation of the DPPH solution [15]. In this study the extract of *Costus speciosus* (thebu) showed the best IC_{50} for the inhibition of the DPPH radicals with a value of 4.63 ±0.13 µg/mL. The plant extracts of *Eleusine indica* (Balathana upper root), *Sida rhombifolia* (Babila upper root) and *Murraya koenigii* (Karapincha) also showed remarkable scavenging activities with IC_{50} of 9.04 ±0.17, 11.0 ±0.17 and 11.67 ±0.29 µg/mL respectively. All other extracts showed IC_{50} values below 500 mg/ml, except for *Coriandrum sativum* (Koththamalli) with an IC_{50} of 842.63 ±0.14 µg/mL. Therefore, these results suggest that all plant extracts carry chemical constituents with the ability of donating hydrogen to a free radical and there by scavenging the potential damage caused by free radicals.

In reducing power assay, we measured the reduction of the yellow colour of the ferricyanide complex (Fe^{3+}) to green color ferrous form (Fe^{2+}) which is monitored by absorbance measurement at 700 nm. Therefore, the reducing property indicates the ability of the extract to exert its antioxidant action by donating a hydrogen to retard the chain reactions of free radicals [16]. The reducing activity of the extracts were compared to that of Gallic acid and *Murraya koenigii* (Karapincha) showed the best reducing power and *Canthium coromandellicum* was the comparatively weakest extract.

Secondary metabolites such as flavonoids, polyphenolic compounds, etc. isolated from plants are derived from phenylalanine and tyrosine [17]. These phenolic compounds with hydroxyl groups have the ability of scavenging most oxidizing molecules, including singlet oxygen, and various free radicals and therefore can act as good antioxidants [18]. Therefore, the food industry use many plant extracts with antioxidant activities to inhibit the lipid peroxidation, to improve the nutritional values and quality of food [19]. These phenolic compounds are also reported to be effective in treating many diseases such as cancer, diabetes, arthritis, as an antiaging agent etc. This study reveles that *Murraya koenigii* (Karapincha) to contain the highest concentration of phenolic compounds with 137.39 ±1.35 GAE mg/g extract.

Murraya koenigii (Karapincha) showed the best total reducing power and also showed the highest TPC in its extract. This plant leaves of *Murraya koenigii* are common in the urban population and however used only in small quantities as a spice leaf. This leaf can be used as a green leaf in larger quantities as a vegetable in various preperations as pastes and soups. *Costus specioses* (thebu) showed the best DPPH scavenging activity of all the plant extracts is reported to be used in many ayurvedic treatments especially for the diabetics. However, *Costus specioses* plant is not commonly found in the urban areas but commonly used in the sub-urban areas. The most common vegetables as *Alternanthera sessilis* (mukunuwenna), *Centella asiatica* (Gotukola) and *Ipomoea aquatic* (Kangkung) showed moderate activities with respect to DPPH radical savenging activity, total phenolic content and total reducing power. However, *Ipomoea aquatic* (Kangkung) showed an IC_{50} of 22.20 ±0.45 for DPPH scavenging activity exhibiting good scavenging properties.

No significant correlation was found in the total phenolic content to the DPPH scavenging activity or the total reducing power. It is reported that the Folin-Ciocalteu method responds differently to various phenolic compounds depending on their chemical structure [20] and also it does not repond to antioxidants such as ascorbic acid [21].

5. Conclusion

Use of natural antioxidants over synthetic drugs can be advantageous due to its cost effectiveness and easy accessibility in the form of vegetables. In the present study analysis of free radical scavenging activity and total phenolic and total reducing power showed that all the test sample to carry good antioxidant activity with *Murraya koenigii* (Karapincha) and *Costus speciosus* (thebu) exhibiting remarkable activities over more popular greens used among the urban population.

6. Tables and Figures

Table 1: Medicinal values of edible plants [11]

Botanical name	Family	Common name (Sinhala)	Part	Medicinal uses
Coccinia grandis	Cucurbitaceae	Kowakka	Leaves	Diabetes, wound, cough, asthma, Swelling
Sida rhombifolia	Malvaceae	Babila	Upper root	Fever, hypertension, edema
Costus speciosus	Zingiberaceae	Thebu	Leaves	Cough, syphilis, worms, skin diseases, diabetes mellitus
Aporosa lindleyana	Euphorbiaceae	Kebella	Leaves/root	Antimicrobial and analgesic
Ipomoea aquatic	Convolvulaceae	Kankun	Leaves, Stems	Diabetes mellitus, ringworm infestation, fever, delirium
Murraya koenigii	Rutaceae	Karapincha	Leaves	Snake bit, nausea, fever, asthma, hypercholesteremia
Coriandrum sativum	Apiceae	Koththamalli	Seed, leave	Gastritis, cough, asthma, pain, fever, nausea, urinary tract infection
Scoparia dulcis	Scrophularia-ceae	Wal koththamalli	Leaves, Stem	Diabetes mellitus, vomiting, ear and eye disease, stomach ailment
Ipomoea batatas	Convolvulaceae	Bathala	Leaves, root	Asthma, miscarriage, nausea, cancer
Alternanthera sessilis	Amaranthaceae	mukunuwenna	Whole, plant	Eye and skin diseases, burning sensation in feet, skin rash
Canthium coromandellicum	Rubiaceae	Kara	leaves	Snake bites, antioxidant, hypocholesterolemic effect
Centella asiatica	Apiaceae	Gotukola	Whole plant	Wound, burn, ulcrative ailment
Olax zeylanica	Olacaceae	malla	leaves	Snake bites, diabetic
Eleusine indica	Poaceae	Balathana	Upper root	Antioxidant, anti-inflammatory

Table 2: Total Phenolic Content (as Gallic acid equivalents) and IC_{50} (µg/ml) of DPPH scavenging activity of edible plant extracts

Botanical name	Family	Common name (Sinhala)	IC_{50}values of DPPH(µg/mL)	Total Phenolic Content (EGA (mg) / extracts(g)
Alternanthera sessilis	Amaranthaceae	mukunuwenna	211.32 ±3.72	125.12±1.13
Aporosa lindleyana	Euphorbiaceae	Kebella	63.13 ±0.20	99.46 ±1.86
Canthium coromandellicum	Rubiaceae	Kara	255.59 ±1.47	22.99 ±0.56
Centella asiatica	Apiaceae	Gotukola	155.00 ±2.77	55.00±0.56
Coccinia grandis	Cucurbitaceae	Kowakka	262.48±3.02	67.54 ±0.26
Coriandrum sativum	Apiceae	Koththamalli	842.63 ±0.14	10.12 ±0.11
Costus speciosus	Zingiberaceae	Thebu	4.63 ±0.13	38.71 ±1.94
Eleusine indica	Poaceae	Balathana	9.04 ±0.17	14.50 ±0.69
Ipomoea aquatic	Convolvulaceae	Kangkung	22.20 ±0.45	37.90 ±0.06
Ipomoea batatas	Convolvulaceae	Bathala	51.19 ±0.29	44.95 ±0.23
Murraya koenigii	Rutaceae	Karapincha	11.67 ±0.29	137.39 ±1.35
Olax zeylanica	Olacaceae	malla	30.88 ±0.62	12.95 ±1.52
Scoparia dulcis	Scrophularia-ceae	Wal koththamalli	109.06 ±0.21	18.17 ±0.23
Sida rhombifolia	Malvaceae	Babila upper root	11.0 ±0.17	10.98 ±0.17
Sida rhombifolia	Malvaceae	Babila root	135.80 ±6.16	3.07 ±0.06

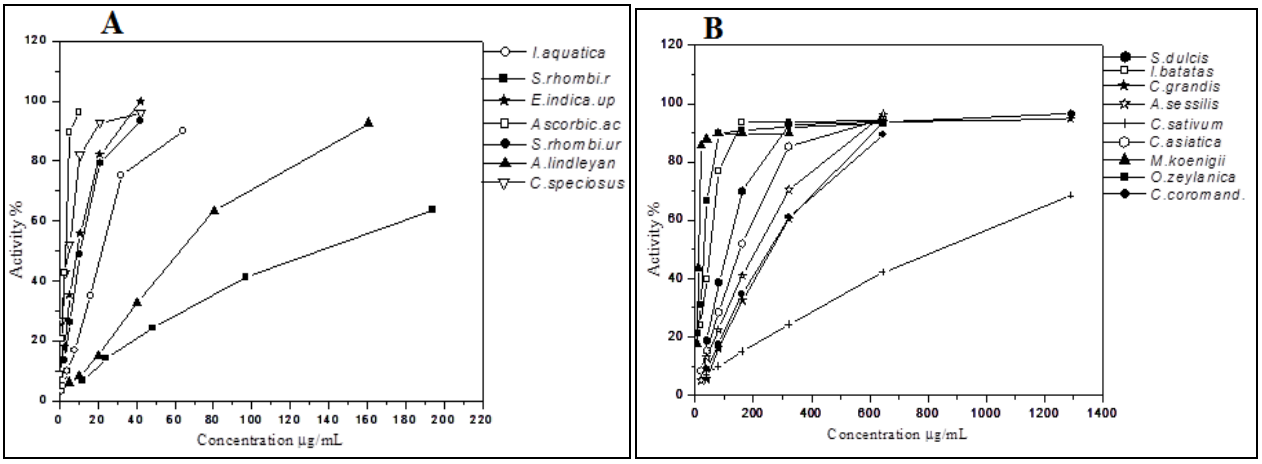

Fig 1: DPPH free radical scavenging activity assay

A-Graph included, *S. aquatica, S. rhombifolia* root, *E. indica* uproot, Ascorbic acid, *S. rhombifolia* up root, *A. lindleyan, C. specious.*
B-Graph included. *S. dulcis, I. batatas, C. grandis, C. sativum, C. asiatica, M. koenigii, O. zeylanica, C. coromand and A. sessilis.*

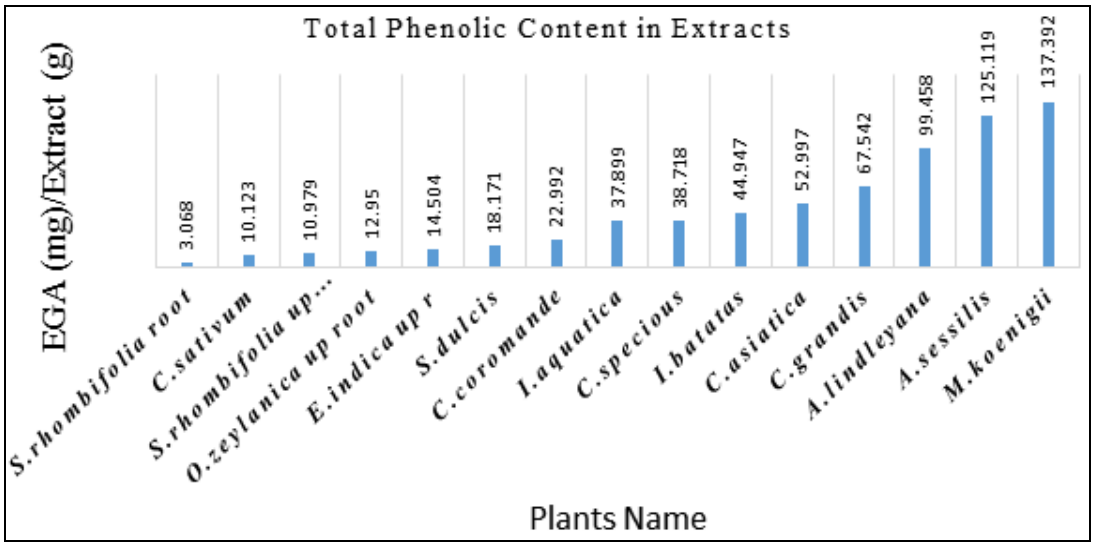

Fig 2: Total Phenolic content of the plant extracts as measured using F-C reagent and express as Gallic acid equivalent (mg) /Extract (mg)

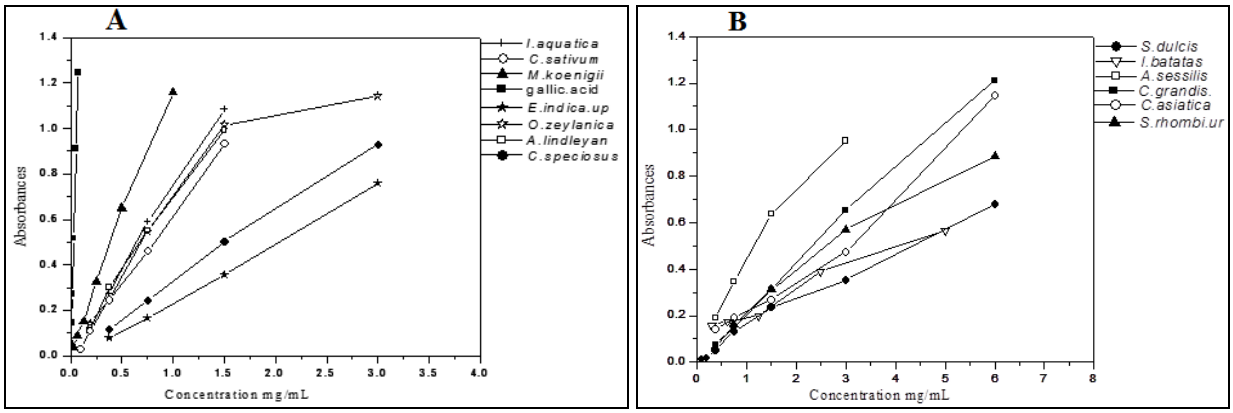

Fig 3: Total reducing power of plant extracts

Graph-A-*I. aquatica, C. Sativum, M. Koenigii*, Gallic. acid, *E. indica (*uproot), *O. zeylanica, A. lindleyan, C. specious*
Graph-B- *S. dulcis, I. batatas, C. asiatica, C. grandis, C. asiatica, S. rhombifolia* up root
**S. rhombifolia root* and *C. coromandel (data not shown)*

7. References

1. Maqsood S, Singh P, Samoon MH, Balange AK. Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. Inter Aqua Res. 2010; 2:77-85.
2. Vongtau HO, Abbah J, Chindo BA, Mosugu O, Salawu AO, Kwanashie HO *et al.* Central inhibitory effects of the methanol extract of *Neorautanenia mitis* root in rats and mice. J Pharm Biol. 2005; 43:113-120.
3. Oluyemi KA, Okwuonu UC, Baxter DG, Oyesola TO. Toxic effects of methanolic extract of *Aspilia africana* leaf on the estrous cycle and uterine tissues of Wistar rats. Int J Morphol. 2007; 25:609-614.
4. Zengin G, Cakmak YS, Guler GO, Aktumsek A. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. *Hayekiana wagenitz*. Rec Nat Prod. 2011; 5:123-132.
5. Gulcin I. Antioxidant activity of food constituents: an overview. Arch Toxicol. 2012; 86:345-391. 10.1007/s00204-011-0774-2.
6. Gocer H, Gulcin I. Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. Int J Food Sci Nutr. 2011; 62:821-825.
7. Nunes PX, Silva SF, Guedes RJ, Almeida S. Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health. 2012.
8. Harman D. Free radical theory of aging. Current status. Amster-dam: Elsevier. 1998, 3-7.
9. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 2006; 97:654-660.
10. Wannes WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G *et al.* Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. Food Chem Toxicol. 2010; 48:1362-1370.
11. Ayurvedic medicinal plants of Sri Lanka http://www.instituteofayurveda.org/plants/
12. Mahesh KN, Wickramaratne MN, Wickramaratne DBM. Evaluation of Antioxidant Activity of Five Medicinal Plants in Sri Lank, Pharmacognosy Journal. 2014; 6(3):49-53.
13. Wickramaratne MN, Gunatilake LP, Anuradha NGD, Godavillathanna A, Perera MGN, Nicholas I. Journal of Pharmacognosy and Phytochemistry. 2105; 4(2):121-126.
14. Umamaheswari M, Chatterjee TK. *In vitro* antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. Afr J Trad Compl Altern Med. 2008; 5:61-73.
15. Krishnaiah D, Sarbatly R, Nithyanandam RR. A review of the antioxidant potential of medicinal plant species. Food Bioprod Process. 2011; 89:217-233.
16. Gordon MH. The mechanism of antioxidant action in vitro. Food antioxidants. Edited by: Hudson BJ, London: Elsevier Applied Science. 1990, 1-18.
17. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A. 2004; 1054:95-111.
18. Bravo L. Polyphenols: chemistry, dietary sources, metabolism and nutritional significance. Nutr Reviews. 1998; 56:317-333.
19. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS. Antioxidant activity of plant extracts containing phenolic compounds. J Agri Food Chem. 1999; (47):3954-3962.
20. Satue-Gracia MT, Heinonen M, Frankel EN. Antioxidant activity of anthocyanin in LDL and lecithin liposome systems. J Agric Food Chem. 1997; 1997(45):3362-3367.
21. Singleton VL, Rossi JA. Colourimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144-158.