Ceylon Journal of Science 48(2) 2019: 155-162
DOI: http://doi.org/10.4038/cjs.v48i2.7619

RESEARCH ARTICLE

Comparison of antioxidant activity, Phenolic and Flavonoid contents of selected medicinal plants in Sri Lanka

W.M.A.P. Wanigasekera[1,*], A. Joganathan[1], R. Pethiyagoda[1], L.N. Yatiwella[1] and H.M.D.A.B. Attanayake[2]

[1]Department of Basic Veterinary Science, Faculty of Veterinary Medicine & Animal Science, University of Peradeniya, Peradeniya, Sri Lanka
[2]Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka

Received: 03/08/2018 ; Accepted: 25/10/2018

Abstract: Oxidative stress related diseases are as a result of accumulation of free radicals in the cellular organs. Plant based antioxidants play a defensive role by preventing the generation of free radicals and therefore the main focus of this study was to screen twenty five Sri Lankan medicinal plants for comparison of antioxidant capacity. Aqueous extracts of twenty five plants were screened for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Six plants that gave high antioxidant activity *Terminalia chebula* Retz., *Punica granatum* L., *Flueggea leucopyrus* Willd., *Cassia fistula* L., *Piper betle* L. and *Phyllanthus amarus* were selected for further analysis. Methanol extracts of the selected plants were subjected to assess IC_{50} by DPPH assay. Total phenolic and flavonoid contents were analyzed by Folin Ceocalteu method and Aluminium Colorimetric method respectively. Statistical analysis was done by Minitab 17 package using regression analysis techniques to identify the effect of IC_{50} by phenolics and flavonoids. Seeds of *T. chebula* Retz. showed high antioxidant activity with 102 mg/ml of IC_{50} value. Antioxidant activity of rest of the plants in the descending order was *F. leucopyrus*Willd. (twigs) > *C. fistula* L. (bark) > *P.granatum* L. (leaves) > *P. betle* L. (leaves) >*P. amarus* (twigs). The relationship between IC_{50} by phenolic and flavonoid content was statistically significant. Further IC_{50} has a strong negative relationship between phenolics and flavonoids. Data from present study revealed that *Terminalia chebula* Retz., *Punica granatum* L., *Flueggea leucopyrus* Willd., *Cassia fistula* L., *Piper betle* L. and *Phyllanthus amarus* possess high antioxidant capacity compared to other medicinal plants.

Keywords : *Terminalia chebula*, *Flueggea leucopyrus*, *Cassia fistula,* DPPH, Phenolics, Flavonoids.

INTRODUCTION

Antioxidants are compounds that prevent oxidation through one or more mechanisms. They scavenge free radicals that can harm the human body. There are many reviews on the relationships between oxidative damages and various diseases including cancer, liver disease, aging, arthritis, diabetes, atherosclerosis, acquired immune deficiency syndrome (AIDS) etc. (Ullah *et al.*, 2016; Tribble *et al.,* 1994; Willcox *et al.,* 2004). As a result many diseases have been treated with antioxidants to prevent oxidative damage. The natural antioxidants, vitamin E, vitamin C, and polyphenols have been investigated for their possible use in preventing above diseases. Phenolic compounds present in plants have been reported as the main contributors to the antioxidant activity of fruits, vegetables, and medicinal plants (Stankovic, 2011). It was reported in France that drinking red wine has been linked to the low incidence of coronary heart disease (CHD), and later it was confirmed that polyphenols, flavonoids and anthocyanins in red wine play an important role in the prevention of CHD (Kanner *et al.,* 1994).

Sri Lanka has different varieties of medicinal plants whose effectiveness has been proven across many generations as herbal treatments for control of diseases. Some of the diseases with complicated etiologies such as cancer, diabetes, arthritis have been recognized to be controlled or cured using these herbal medicines. Antioxidants present in plants may play a major role in controlling these diseases (Ayurvedic Medicinal plants of Sri Lanka, 2017). There were many reports on antioxidant activity of medicinal plants but antioxidant activities have not been compared among each other. Therefore aim of this study was to compare antioxidant capacity of medicinal plants that are extensively used in traditional medicines. In the present investigation, twenty five medicinal plants were screened by 2,2-diphenyl-1-picrylhydrazyl(DPPH) assay. Plants that showed high antioxidant activity *Terminalia chebula* Retz, *Punica granatum* L., *Flueggea leucopyrus* Willd., *Cassia fistula* L., *Piper betle* L. and *Phyllanthus amarus* were selected to determine IC_{50} by DPPH assay, total polyphenol content and flavonoid content.

MATERIALS AND METHODS

Chemicals

Aluminum Chloride, Sodium Nitrite, Sodium Carbonate were purchased from Dea Jung South Korea. DPPH and Folin Ciocaltue were obtained from Sigma Chemicals. Gallic acid and Quercetin were purchased from Sisco Research Laboratories. All other solvents and chemicalswere of analytical grade.

**Corresponding Author's Email:* anojapw@gmail.com;anojapw@pdn.ac.lk
http://orcid.org/0000-0002-8087-0027

Selection and collection of the plant materials

Twenty five medicinal plants were selected by following discussions with several local Ayurveda practitioners and based on literature survey. The scientific names, vernacular (Sinhala) names and the plant parts selected are detailed in Table 1. The plant part used are the same as those generally used in medicinal preparations in the traditional Sri Lankan medicine such as leaves, stems/ bark, fruits, seeds, rhizomes or whole plant. For the initial screening of medicinal plant extracts for antioxidant capacity, all the plant materials were bought from reputed indigenous medicinal shops in Kandy and were verified by a local physician. Plants that showed high antioxidant activity by DPPH assay were collected freshly with the permission of garden owners from different localities of Sri Lanka. Bark of *C. fistula* and the seeds of *T. chebula* were collected from Moneragala (Uva Province). Twigs of *F. leucopyrus* were collected from Rantambe (Central Province). *P. amarus*was collected from Hemmathagama (Sabaragamuwa Province) and leaves of *P. granatum* and *P. betle* were collected from Udaperadeniya (Central Province). Plants were authenticated by comparison with the herbarium specimens at the National Herbarium, Department of National Botanic Gardens, Peradeniya, Sri Lanka. A voucher specimen of each plant is deposited at the National Herbarium (Deposition Number: 6/01/H/03).

Preparation of water extract of plants for antioxidant activity

Plant material was washed with distilled water to clean off dirt and dust. They were cut into small pieces and dried in an oven at 30^{o}C until constant weight was attained. Dried samples were pulverized using a domestic blender. Each powdered sample was transferred separately into an air-tight bag and was stored in a refrigerator (-4^{o}C) until extraction. Dried powdered plant material (0.5g) was transferred into a screw-capped test tube, together with 10.0 ml of distilled water and incubated at 95^{o}C in a water bath for 1 hour. The resulting water extract was centrifuged at 3900 rpm for 5 minutes and the supernatant was stored in a freezer at-20^{o}C for further analysis.

Screening of water extracts for free radical scavenging activity by DPPH assay

This assay is based on the determination of the concentration of DPPH solution, after adding the antioxidants. An aliquot (40 µl) of plant extract was added to 3 ml of 8 x10^{-5} M DPPH and samples were incubated at room temperature for 20 minutes in the dark. Absorbance wasmeasured at 517

Table 1: Scientific name, vernacular names andplant parts.

Scientific Name	*Vernacular names	Plant part
*Asparagus racemosus*Willd.	Hathawariya	leaves
Azadirachta indica A. Juss.	Kohomba	Bark
Boerhavia diffusa L.	Saarana	roots
Cardiospermum halicacabum L.	WelPenela	whole plant
Cassia fistula L.	Ehela	Bark
Coriandrum sativum	Koththamalli	Seeds
Coscinium fenestratum	Weniwelgeta	Stem
Cyperus rotundus	Kalanduru	rhizomes
Fluggea leucopyrus Willd.	Katupila	twigs
Kalanchoe pinnata	Akkapana	leaves
Mollugo cerviana	Pathpadagam	whole plant
Munronia pinnata L.	BimKohomba	whole plant
Pavetta indica L.	Pavatta	Stem
Phyllanthus amarus	Pitawakka	whole plant
Piper betle	Bulath	leaves
Piper longum L.	Thippili	Fruit
Pterocarpu ssantalinus L.	RathHandun	Stem
Punica granatum	Delum	leaves
Solanam trilobatum L.	WelThibbotu	whole plant
Solanum virginianum L.	KatuwelBatu	whole plant
Sphaeranthus indicus L.	Mudumahana	whole plant
Terminalia chebula Retz.	Aralu	seeds
Tinospora cardifolia	Rasakinda	stem
Vetiveria zizanioides	Savandara	roots
Zingiber officinale Roscoe.	Inguru	rhizomes

*Commonly used Sri Lankan (Sinhala) names

nm using UV–Vis spectrophotometer (GBG Cintra-6). All samples were analyzed in triplicate and results averaged. Antioxidant activity of plant extracts was expressed as mmols of ascorbic acid equivalents per gram of plant material on a dry basis [Wong *et al,* 2006].

Preparation of methanol extract for antioxidant activity

All fresh samples were washed with distilled water and air dried at room temperature. Plant parts were cut into small pieces and air dried until constant weight was obtained. Dried samples were powdered using a domestic grinder, packed in air tight polyethylene bags and stored at -4°C until use for analysis. Powdered plant materials (20 g)were extracted with 100 ml of methanol in a screw capped conical flask with occasional shaking at room temperature. Plant residue was re-extracted twice with 100ml of methanol. The mixture was filtered and evaporated using a rotary evaporator (Buchi RII) at 40°C to obtain crude extracts. Extract was stored at -4°C for further analysis.

Determination of free radical scavenging activity of methanol extract by DPPH assay

DPPH assay for total antioxidant capacity was carried out as described in Brand-Williams *et al*, 1995. Reaction mixture was prepared with 0.98 ml of 8 $x10^{-5}$ M DPPH and 20 ml of plant extract at concentrations ranging from 0.016 to 2 g/l. Ascorbic acid was used for comparison or as positive control. All samples were incubated at room temperature for 20 minutes in dark and then absorbance was measured at 517 nm. Measurements were performed in triplicates. The percentage of DPPH radical scavenging activity was determined using the formula:

$$\% \text{ Inhibition} = [A_{(DPPH)} - A_{(Sample)} / A_{(DPPH)}] \times 100$$

where $A_{(DPPH)}$ was absorbance of DPPH control solution and $A_{(Sample)}$ was absorbance of DPPH solution in the presence of plant extract. Sample concentration giving 50% inhibition was estimated as IC_{50} value using the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging activity.

Determination of total polyphenol content using Folin-Ciocaltue reagent

The concentration of phenolic in plant extracts was determined using spectrophotometric method (Singleton *et al,* 1999; Waterhouse, 2002). Aliquot of (20 µl) gallic acid calibration standards were mixed with 1.58 ml of distilled water and 100 µl of Folin-Ciocalteu's reagent. The reaction mixture was incubated at room temperature for 8 min (not exceeding 8 minutes) and then 300 µl of 200mg/ml sodium carbonate was added, re-incubated for another 2 hours at room temperature and absorbance was measured at 765 nm. Analysis was performed in triplicate and the average absorbance was used to plot the calibration graph. The same procedure was repeated for the plant extracts. Based on the measured absorbance, the concentration of phenolics were read (mg/ml) from the calibration graph and the content of phenols was expressed in terms of gallicacid equivalents per gram of extract (mg of GAE/g). Data are expressed as mean± SD of three replicates.

Determination of flavonoid content by Aluminium Chloride Colorimetric assay

Total flavonoid content was determined by Aluminium colorimetric method (Nunes *et al.,* 2012; Thangaraj *et al*., 2016). Quercetin (QE) calibration standards (500µl) were pipetted in to 5ml volumetric flasks with 2ml of distilled water and 0.15ml of 5% $NaNO_2$, vortex thoroughly, and incubated at room temperature for 5 minutes. After incubation, 0.15ml of 10 % $AlCl_3$ was added to each mixture, except for the blank to which same volume of distilled water was added, and incubated again at room temperature for 6 minutes. 1ml of 4% NaOH was added to the mixture, vortexed and made the volume up to 5ml with distilled water. Absorbance was measured at 430 nm after 15minutes. All dilutions were analyzed in triplicate and average absorbance was used to plot the calibration graph. The standard graph was drawn with absorbance against the concentration of QE dilutions. Data are expressed as mean± SD of three replicates. Same procedure was repeated with the plant extract and the content of the flavonoids was expressed in terms of QE equivalents per gram of plant extract.

Statistical analysis

All graphs were drawn using Microsoft Excel. Data were expressed as means, and standard deviation. Statistical analysis was done by Minitab 17 statistical package using Regression analysis techniques to identify the effect of IC_{50} by phenolics and flavonoids.

RESULTS AND DISCUSSION

Antioxidant activity of plant extracts by DPPH assay

There are various methods to evaluate antioxidant capacity. DPPH analysis is one of the accurate and frequently used methods for evaluating antioxidant capacity (Pérez-Jiménez *et al.,* 2008). DPPHis a stable free radical due to delocalization of the extra electron on the whole molecule and therefore DPPH does not dimerize as it happens with other free radicals. This property gives rise to a deep violet colour for DPPH that can absorb UV light at about 517 nm (Sharma and Bhat, 2009). Antioxidants react with DPPH radical, forming reduced form of DPPHand the intensity of the resulting colour is proportional to the remaining concentration of DPPH after reaction with the antioxidant (Molyneux, 2004).

The results of antioxidant activity of water extracts by DPPH assay are given in Figure 1. Antioxidant activity was expressed as ascorbic acid equivalents per gram of plant material on a dry basis (Wong *et al.,* 2006). Out of twenty five samples analyzed, six had high antioxidant capacity (> 0.5 mmol/g dry weight of sample). *T. chebula,* Retz. gave the highest antioxidant activity of 5.41 mmol/g of dry weight. The order of radical scavenging capacity as *T. chebula* > *P. granatum* > *F. leucopyrus* Willd. > *C. fistula* > *P. betle* > *P. amarus*. The results of antioxidant activity of methanol extracts at different concentrations, estimated by DPPH assay are given in Figure 2. The concentration of

sample required to give 50% scavenging activity of DPPH free radical is considered as the total antioxidant activity. IC_{50} value was determined from the plotted graphs and is shown in the Table 2. According to the results, methanol extract of *T.chebula* Retz. showed high total antioxidant activity with IC_{50} value of 102 mg/l(r^2= 0.98) compared to the other plants. The IC_{50} of *F. leucopyrus* Willd. (r^2= 0.97), *C. fistula* L.(r^2= 0.98), *P. granatum* (r^2= 0.99), *P. betle* (r^2= 0.98)and *P. amarus* (r^2= 0.99) were 353 mg/ l,489 mg/ l, 508 mg/l, 623 mg/l and 1037 mg/lrespectively. Ascorbic acid was used as the reference standard and IC_{50} value was 3.18 mg/L (r^2= 0.98).

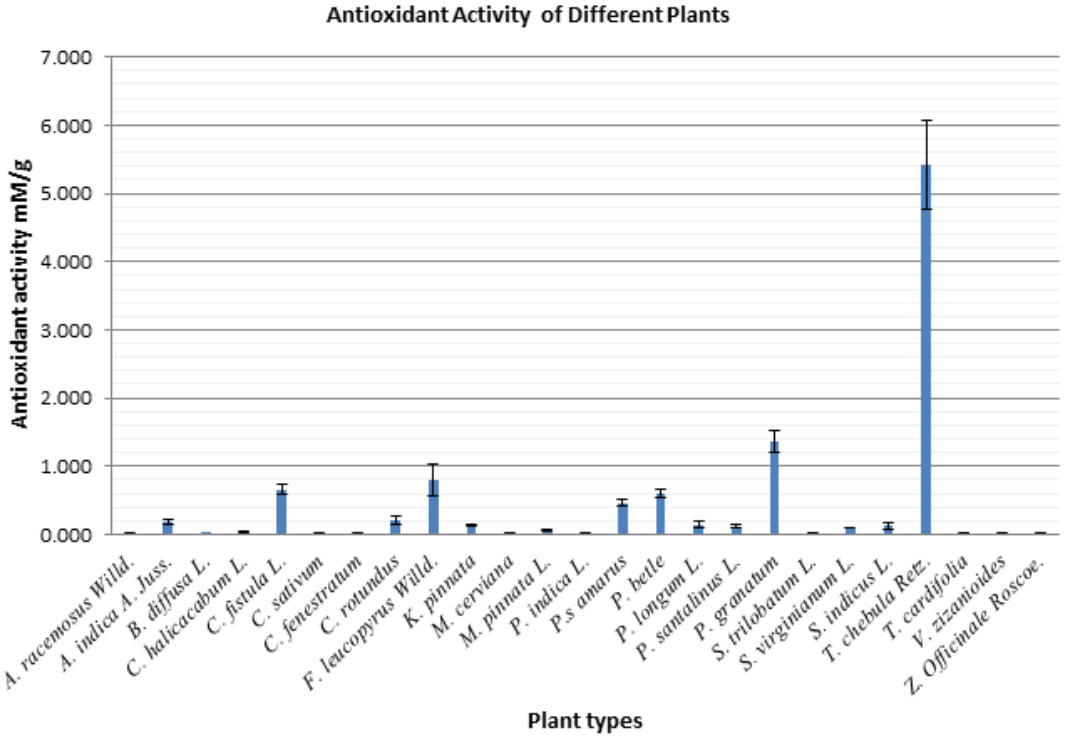

Figure 1: DPPH Radical scavenging activity of water extract of 25 medicinal plants (n=3 each, error bars represent standard deviation) expressed as ascorbic acid equivalents per gram of plant material on a dry basis.

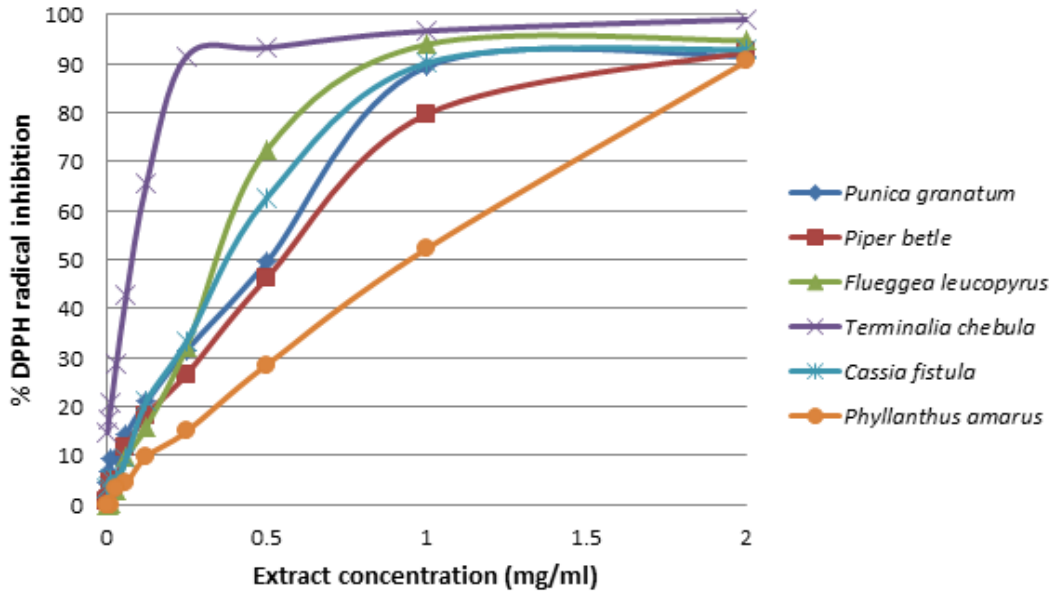

Figure 2: DPPH free radical scavenging activity at different concentrations of methanol extracts of *Cassia fistula, Flueggea leucopyrus, Phyllanthus amarus, Piper betle, Punica granatum* and *Terminalia chebula*.

Total phenol content by Folin- Ciocaltue reagent

Phenolic compounds are a group of antioxidants that act as free radical terminators. The amount of total phenol was determined using Folin Ciocalteu reagent (Brand-Williams *et al.,* 1995). This test is based on the oxidation of phenolic groups by phosphomolybdic and phosphotungstic acids (Folin-Ciocaltue) and yielding a blue colour with a broad maximum absorption at 765 nm (Singleton *et al.,* 1999; Agbor and Vinson, 2014). Gallic acid was used as a standard compound and total phenols were expressed as mg/g of gallic acid equivalents. Figure 3 shows the standard graph of Gallic acid. Total phenols were expressed as mg/g of gallic acid equivalents using the standard graph equation:y = 0.8243x + 0.0135, R^2 = 0.99, where y is absorbance at 765 nm and x is total phenolic content. Table 3 shows the total phenolcontent that was measured by Folin Ciocaltue reagent in terms of gallic acid equivalent. *T. chebula* showed high total phenol content of 121.72 mg/g. Total phenol content of plant extracts reduces in the order of *T. chebula* > *C. fistula* > *F. leucopyrus* > *P. granatum* > *P.betle* >*P. amarus*. The relationship between IC_{50} (mg/l) by phenolic contents was statistically significant ($p < 0.05$). Further, IC_{50} has a strong negative relationship between phenolics, the correlation value (r) of -0.82 with antioxidant activity (Figure 4).

Table 2: Radical scavenging capacity of crude methanolic extract of *C. fistula, F. leucopyrus, P. amarus, P. betle, P.granatum* and *T. chebula*.

Name of the plant	DPPH (IC_{50}±SD) mg/l
Cassia fistula Linn.	489 ± 10
Flueggea leucopyrus Willd.	353 ± 15
Phyllanthus amarus	1037 ± 11
Piper betle	623± 40
Punicag ranatum L.	508 ± 9
Terminalia chebula Retz	102 ± 4

Each value in the table is represented as mean ± SD (n = 3).

Table 3: Total phenolic content and flavonoid content.

Name of the plant	Total phenolic content mg/GAE/g extract	Total flavonoid content mg/QE/g extract
Cassia fistula Linn.	43.57 ±1.16	4.99 ± 0.10
Flueggea leucopyrus Willd.	39.17±0.74	4.58 ± 0.09
Phyllanthus amarus	11.30± 0.38	3.64 ± 0.08
Piper betle	14.83±0.06	4.43 ± 0.03
Punica granatum L.	29.97±0.57	5.48 ± 0.04
Terminalia chebula Retz	121.72±6.04	8.31 ±0.43

Each value in the table is represented as mean ± SD (n = 3).

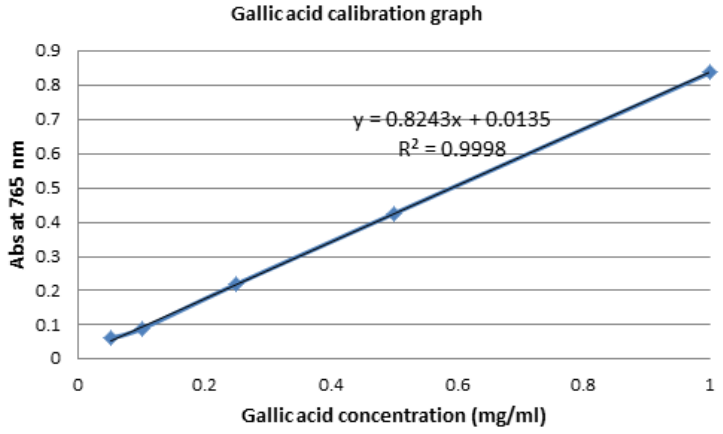

Figure 3: Standard calibration graph of gallic acid.

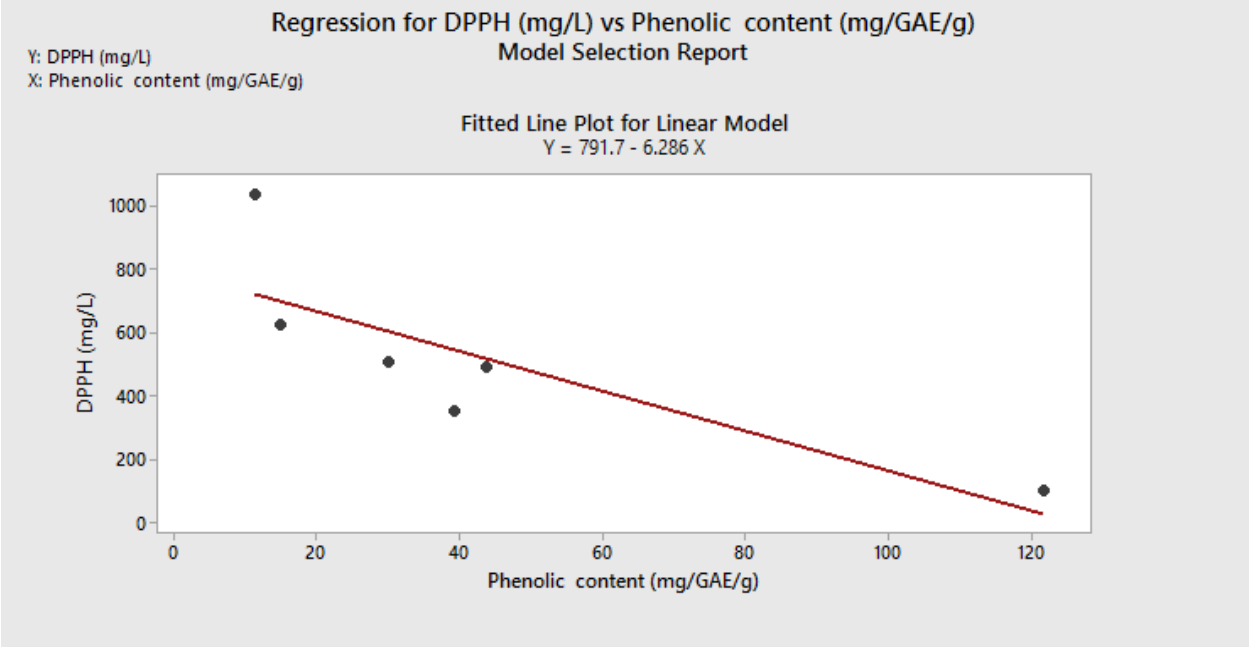

Figure 4: Linear correlation between the amount of total phenol and antioxidant activity (IC_{50} of DPPH).

Total flavonoid content by Aluminium chloride colorimetric method

Total flavonoid content was estimated by Aluminium chloride ($AlCl_3$) colorimetric method. $AlCl_3$ forms acid stable complexes with C-4 keto groups and either C-3 or C-5 hydroxyl group of flavones and flavonols (Thangaraj, 2016, Bhaigyabati *et al.,* 2014). The content of flavonoids was expressed as mg/g of quercetin equivalents. Figure 5 shows the standard graph and the equation is, $y = 1.112x - 0.0164$, $R^2 = 0.9977$, where y is absorbance at 430 nm and x is total flavonoid content. Table 3 shows the mean ±SD of flavonoid content in terms of quercetin equivalent. The high content of flavonoids was observed in *T. chebula* as 8.31 ±0.43 mg/quercetin/g. The relationship between IC_{50} (mg/L) by flavonoid contents was statistically significant ($p < 0.05$). IC_{50} has a strong negative relationship with flavonoids, which gave a correlation value(r) of -0.82 with antioxidant activity (Figure 6).

Antioxidant activity of fruits of *Terminalia chebula* has been tested in many different assays. Harza *et al.,* 2010 reported that IC_{50} of seeds by DPPH as 1.73 µg/ml, comparatively higher antioxidant capacity than our results. High phenolic and flavonoid contents were observed as 127.6 mg Gallic acid equivalent per 100 mg plant extract and 219.30 mg quercetin equivalent per 100 mg plant extract respectively. In *Flueggea leucopyrus,* Soysa *et al* 2014 reported that the phenolic content was 22.15 µg/g of Gallic acid equivalents and DPPH radicals by 50% was 11.16 ± 0.45 µg/mL. There were many reports on the antioxidant activity of different parts of *Cassia fistula*. According to Ilavarasan *et al.,* 2005, IC_{50} of the methanol extract of bark of *Cassia fistula* was 203 µg/mL by DPPH assay. Though there were many reports on antioxidant activity by different assays, a remarkable differences in the values and cannot be compared each other.

Medicinal value of plants

Terminalia chebula is a perennial medicinal plant used widely in indigenous and ayurvedic medicinal systems (Ayurveda Department, 2002], mainly for the treatment of asthma, sore throat, vomiting, hiccough, diarrhea, bleeding piles, gout, heart and bladder diseases (Cheng *et al.,* 2003) and cancer (Saleem *et al.,* 2002). Juice of *P. granatum* has been used to treat sore throats, cough, urinary infections, skin disorders and arthritis (Bhowmik *et al.,* 2013). However modern research suggests that pomegranates might be useful in treating cancer, osteoarthritis and diabetes (Ephraim and Robert, 2007). Limited work has been reported from the leaves of pomegranate, but leaves use in eye infections, dysentery, worm diseases, asthma in ayurvedic medicine in SriLanka (Ayurvedic Medicinal plants of Sri Lanka, 2017) *F. leucopyrus* Willd. is extensively used in the treatment of cancer and tumors in traditional Ayurvedic medicinal practice in Sri Lanka (Soysa *et al.,* 2014) *C. fistula* L. is an important medicinal plant belonging to family Caesalpiniaceae. Stem-bark is useful in the treatment of many diseases such as skin diseases, inflammatory diseases, rheumatics, and jaundice (Bahorun, *et al.,* 2005). *Piper betle* leaves are used in folk medicine for the treatment of various disorders and is commonly chewed among Asians. The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice (Bahorun *et al.,* 2012). Aerial parts of the herb *P. amarus* have been widely used in the treatment of jaundice, constipation, diarrhea, kidney ailments, ringworm, ulcers, malaria etc. (Lim and Murtijaya, 2007).

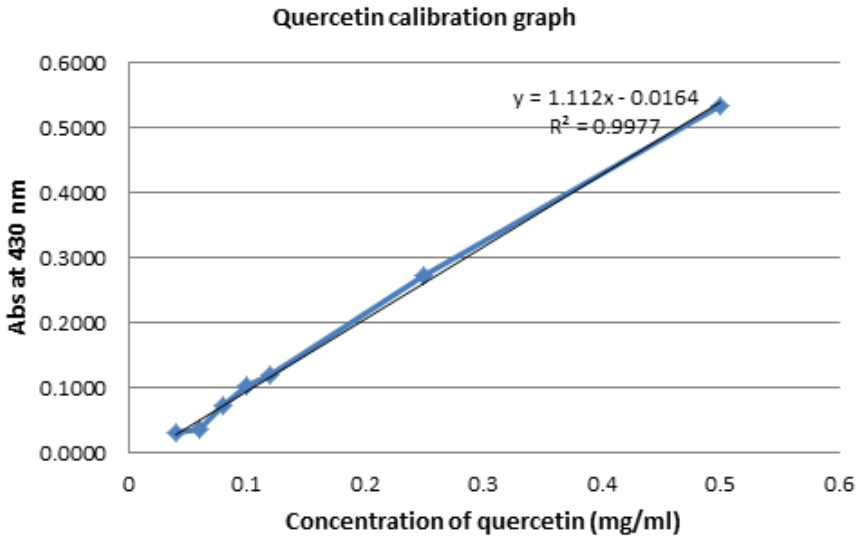

Figure 5: Standard calibration graph of quercetin.

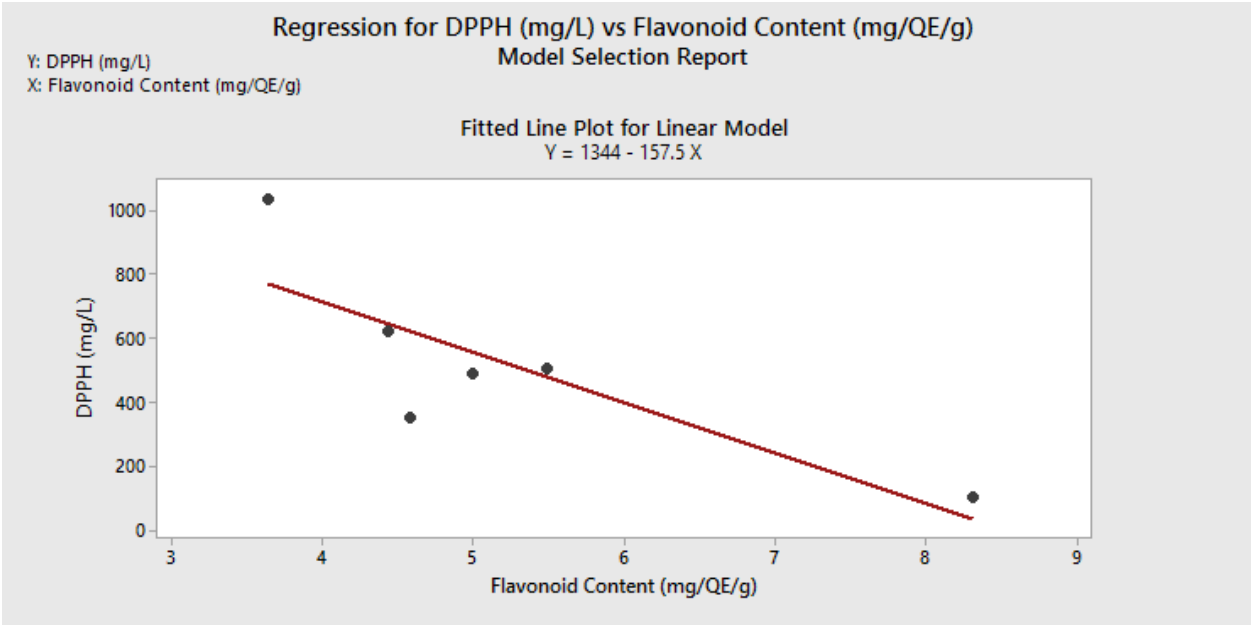

Figure 6: Linear correlation between the amount of flavonoids and antioxidant activity (IC_{50} of DPPH).

CONCLUSIONS

Natural products have been proven relatively safe for human consumption and the plants have been used for thousands of years in the treatment of different diseases. The wide use of these plants in the Sri Lankan indigenous systems may be inpart due to their antioxidant potency. As shown in terms of other antioxidant assays, plants used in this medicinal system convey a very high antioxidant potential. The mechanism of action of these plants require further study and isolation of the secondary metabolites responsible for the antioxidant activity has to be taken up which may result in the discovery of modern drugs from these plants.

LIST OF ABBREVIATIONS

DPPH- 2,2-diphenyl-1-picrylhydrazyl

AIDS - acquired immune deficiency syndrome

CHD- coronary heart disease

ACKNOWLEDGEMENTS

Authors wish to acknowledge RG/2012/56/V and RG/2014/46/V grants of the University of Peradeniya, Sri Lanka for financial support and Mrs. N.P.T. Gunawardena, officer in-charge, National Herbarium, Department of National Botanic Gardens, for authentication of plant specimen.

REFERENCES

Abrahim, N.N., Kanthimathi, M.S. and Abdul-Aziz, A. (2012) *Piper beetle* shows antioxidant activities, inhibits MCF-7 cell proliferation and increases activities of catalase and superoxide dismutase. *Complementary and Alternative Medicine*; **12**:220.

Agbor, G., Vinson, J.O. and Donnelly, P.E. (2014) Folin-Ciocalteau Reagent for Polyphenolic Assay. *International Journal of Food Science, Nutrition and Dietetics.*; **3**(8):147-156.

Ayurveda Department (2002). *Compendium of medicinal plants: A Sri Lankan study.***2**:233-237.

Ayurvedic Medicinal plants of SriLanka. http://www.instituteofayurveda.org/ plants/ plants_detail.php?i=1091&s=Family_name. Accessed 20/08/2017**.**

Bahorun, T., Vidushi, S., Neergheen, V.S. and Aruoma, O. I., (2005) Phytochemical constituents of *Cassia fistula*. *African Journal of Biotechnology* **4**(13):1530-1540.

Bhaigyabati, T. H., Devi, P. G. and Bag, P. G. C. (2014) Total flavonoid content and antioxidant activity of aqueous rhizome extract of three hedychium species of Manipur Valley. *Research Journal of Pharmaceutical, Biological and Chemical Science*; **5**(5):970-976.

Bhowmik, D., Gopinath, H., Kumar, B.P., Duraivel, S., Aravind, G. and Sampath Kumar, K.P., (2013) Medicinal Uses of *Punica granatum* and Its Health Benefits. *Journal of Pharmacognosy and Phytochemistry* **1**(5):28-35.

Brand-Williams, W., Cuvelier, M. E. and Berset, C., (1995) Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft and Technologie* **28**:25-30.

Cheng, H.Y., Lin, T.C., Yu K.H., Yang, C.M. and Lin, C.C. (2003) Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biol Pharm Bull* **26**:1331-1335.

Ephraim, P.L. and Robert, A.N. (2007) *Punica granatum* (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology* **109**(2):177-206.

Hazra, B. Sarkar, R., Biswas, S. and Mandal, N. (2010) Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula, Terminalia belerica* and *Emblica officinalis*. *BMC Complementary and Alternative Medicine* **10**:20.

Kanner, J., Frenkel, E., Granit, R., German, B. and Kinsella, J. E. (1994) Natural antioxidants in grapes and wines.*J. Agric. Food Chem*. **42**(1):64-69.

Lim, Y. Y. and Murtijaya, J.,(2007) Antioxidant properties of *Phyllanthus amarus* extracts as affected by different drying methods. *LWT Food Science & Technology.***40**:1664-1669.

Molyneux, P. (2004) The use of stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci.Technol*. **26** (2):211-219.

Nunes, X.P., Silva, F.S., Almeida, J.R. G.S., Lima, J.T., Ribeiro, L.A.A., Junior, A.L.J.Q. and Filho, J.M.B. (2012) Biological oxidations and antioxidant activity of natural products. *Phytochemicals as Nutraceuticals* - Global Approaches to Their Role in Nutrition and Health. DOI: 10.5772/26956 .

Pérez-Jiménez, J. S., Arranz, M., Tabernero, M. E., Diaz-Rubio, J., Serrano, I., Goñi, A. and Saura-Calixto, F. (2008) Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: extraction, measurement and expression of results. *Food Research International*. **41**(3):274-285.

Ilavarasana, R., Mallikab, M. and Venkataramanc, S. (2005) Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn. bark extracts. *Afr. J. Trad. CAM.* **2**(1):70-85.

Saleem, A., Husheem, M., Haekonen, P. and Pihlaja, K. (2002) Inhbition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz. fruit. *Journal of Ethnopharmacology.* **8**:327-336.

Sharma, O., P. and Bhat. T. K. (2009) DPPH antioxidant assay revisited. *Food Chemistry*. **113**:1202-1205.

Singleton, V.L., Orthofer, R. and Lamuela-Reventos, R.M. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol* **299**:152-178.

Soysa, P., De Silva, I.S. and Wijayabandara, J. (2014) Evaluation of antioxidant and antiproliferative activity of *Flueggea leucopyrus* Willd. (katupila). *BMC Complementary and Alternative Medicine* **14**:274.

Stankovi, M.S.,(2011) Total Phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J. Sci.*; **33**:63-72.

Thangaraj, P. (2016) Pharmacological Assays of Plant-Based Natural Products. *Springer International Publishing AG Switzerland*. DOI 10.1007/978-3-319-26811-8_1.

Tribble, D.L. and Frank E., (1994) Dietary antioxidants, cancer, and atherosclerotic heart disease. *Western journal of medicine* **161**:605-612.

Ullah, A., Khan, A. and Khan, I. (2016) Diabetes mellitus and oxidative stress - A concise review. *Saudi Pharmaceutical Journal* **24**;547-553.

Waterhouse, A., (2002). *Current protocols in food analytical chemistry*. 1st edition. New York: John Wiley.

Willcox, J.K., Ash, S.L. and Catignani, G.L.,(2004) Antioxidants and Prevention of Chronic Disease. *Critical Reviews in Food Science and Nutrition* **44**: 275-295.

Wong, S.P., Leong, L.P. and Koh, J.H.W. (2006) Antioxidant activities of aqueous extracts of selected plants. *Food Chemistry* **99**(4):775-783.