Research article

Antioxidant activity of some Sri Lankan endemic medicinal plants

Weerasinghe W.P.N.W, Deraniyagala S.A.

Department of Chemistry, University of Colombo, PO 1490, Colombo, Sri Lanka

*Corresponding author: sd@chem.cmb.ac.lk

Abstract

Despite the advances observed in modern medicine in recent times, plants still make an important contribution to healthcare. Recent studies have highlighted the importance of herbal medicine in developing antioxidants.

Antioxidant capacities of nine endemic plants (*Garcinia quaesita, Garcinia zeylanica, Dialium ovoideum, Horsfieldia iryaghedhi, Vernonia zeylanica, Dipterocarpus zeylanicus, Argyreia populifolia, Canarium zeylanicum, Plectranthus zeylanicus*) in Sri Lanka were determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the reducing power assay. Total phenolic contents of the plant extracts were determined by the Folin-Ciocalteu assay.

The radical scavenging activities of the methanolic extracts of plants were, G. quaesita (95±1%), *H. iryaghedhi* (94.9±0.2%), *G. zeylanica* (93.3±0.1%), *D. ovoideum* (92.9±0.4%), *D. zeylanicus* (91.1±1%), *V. zeylanica* (90±1 %), *A. populifolia* (77±9 %), *P. zeylanicus* (75.4±0.4%), *C. zeylanicum* (63±1%). The Fe3+ reducing abilities, of the methanolic extracts of plants were, G. *quaesita* (1.99±0.003), *H. iryaghedhi* (1.95±0.01), *G. zeylanica* (1.87±0.08), D. ovoideum (1.44±0.05), *V. zeylanica* (0.35±0.01), *D. zeylanicus* (0.18±0.01), *A. populifolia* (0.11±0.02), *P. zeylanicus* (0.097±0.007), *C. zeylanicum* (0.09±0.007). Folin-Ciocalteu assay results showed the following order. *G. quaesita* (293±3 mg (PGE)/g), *G. zeylanica* (274.5±16.8 mg (PGE)/g), *D. ovoideum* (192.9±11.4 mg (PGE)/g), *H. iryaghedhi* (148.8±12.9 mg (PGE)/g), *V. zeylanica* (80.8±1.2 mg (PGE)/g), *D. zeylanicus* (64.4±2.1 mg (PGE)/g), *A. populifolia* (53.9±5.6 mg (PGE)/g), *C. zeylanicum* (49.2±3.0 mg (PGE)/g), *P. zeylanicus* (34.8±3.2 mg (PGE)/g).

This study shows that these endemic plants, specially, *G. quaesita, H. iryaghedhi, G. zeylanica* and *D. ovoideum* have a high potential to be developed as antioxidants which could serve as dietary supplements.

Key words: Antioxidant capacity; methanolic extracts; DPPH radical scavenging assay; Folin-Ciocalteu assay; Reducing power assay.

Introduction

Despite the advances observed in modern medicine in recent times, plants still make an important contribution to healthcare. This may well be due to the fact that medicines derived from plants are safer than their synthetic counterparts. Recent studies have highlighted the importance of herbal medicine in developing antioxidants.[1]

As a result of the reactions occurring in a body of a living organism, reactive oxygen species and other free radicals are generated which could cause harmful effects to the organism.[2] It has been identified that the free radical reactions are involved in many disorders such as atherosclerosis, diabetes and immunosuppression.[3] Furthermore, the role of free radicals in the process of aging is also well known.[4] Thus, special attention is drawn towards the antioxidants, which have the capability to scavenge free radicals, preventing the possible destructive processes.[2] Therefore,

antioxidants have been recognized as species which could be used in the prevention of diseases and in the maintenance of human health.[1] Since, antioxidants are recommended as dietary supplements[4], there is a considerable interest in search of effective plant based drugs with minimal side effects. Nine endemic medicinal plants in Sri Lanka {*Garcinia quaesita* (Rath Goraka), *Garcinia zeylanica* (Kaha Goraka), *Dialium ovoideum* (Gal Siyambala), *Horsfieldia iryaghedhi* (Ruk), *Vernonia zeylanica* (Pupula), *Dipterocarpus zeylanicus* (Hora), *Argyreia populifolia* (Girithilla), *Canarium zeylanicum* (Kekuna), *Plectranthus zeylanicus* (Iriweriya)} used in folklore medicine[5] for many different ailments, which have not been investigated for their antioxidant activities, were selected for this study.

Methods

Collection of plant material

Fresh plant parts were collected from different areas of Gampaha district, Sri Lanka and the plants were identified and authenticated at the Department of Plant Sciences, University of Colombo. Voucher specimens have been deposited in the Advanced Chemistry Laboratory, Department of Chemistry, University of Colombo. The collected parts of the plants are as shown in Table 1. All the samples were washed and air dried for two weeks. Dried plant materials were ground to powder and they were placed in sealed plastic containers until extraction. Preparation of the extracts from the plant materials, 0.5 g of samples were measured using an analytical balance (OHAUS, PA313) and they were mixed with 10 ml of distilled methanol. Using an ultrasonic bath (Grant Instruments (Cambridge) Ltd, Model No: XUB12), extraction was carried for 1 hour and the samples were centrifuged (Spectrafuge 6c compact , Labnet International Inc., Serial No: c406476) for 10 min at 2500 rpm. The supernatants were collected and kept in the refrigerator for analysis.[6] The extracted samples were used for analysis within 12 hours of extraction.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay[1,6]

A portion of 3.9 cm^3 from a 24 mg/dm^3 DPPH solution was mixed with 100 µl of the plant extract. Blank was prepared by mixing 100 µl of plant extract with 3.9 ml of methanol. DPPH solution was taken as the control. Absorbance of all the samples was measured (Spectrum SHANGHAI, KJ0110020429) at 516 nm after 30 min of mixing. The percentage radical scavenging activity (RSA) was calculated according to equation 1 shown below.[1] The experiment was done in triplicates.

Reducing Power Assay[7]

From each plant extract sample, 100 µl was mixed with 1.9 ml of distilled water. To this solution 2.0 ml of phosphate buffer (0.2 mol/dm^3, pH 6.6) and 2.0 ml of potassium ferricyanide (1% w/v) solution were added. The mixture was kept at 50 °C in a water bath for 20 min. After cooling, 2.0 ml of 10% trichloro acetic acid solution was added and the mixture was centrifuged at 3000 rpm for 10 min. An aliquot of 2.0 ml from the supernatant was mixed with 2.0 ml of distilled water and 0.4 ml of freshly prepared $FeCl_3$ solution (0.1%). After 10 min, absorbance was measured at 700 nm using a spectrophotometer (Spectrum SHANGHAI, KJ0110020429). Same procedure was carried out using 100 µl of distilled water instead of the plant extract and it served as the blank.

Folin-Ciocalteu Assay[8]

An aliquot (200 µl) of the diluted extracts were mixed with 2% sodium bicarbonate (4 ml) and was incubated in dark for 2 min. A portion of 200 µl of the Folin-Ciocalteu reagent was added and the mixture was again incubated in dark for 30 min. The absorbance was measured at 750 nm. Blank was prepared by replacing the 200 µl plant extract with methanol. To prepare the standard curve, the same procedure was carried out with 200 µl of pyrogallol instead of plant extracts.

Statistical analysis

All the experiments for each sample were

carried out in triplicates and the results are presented in mean ± standard deviation (SD) using Microsoft excel 2013.

Equation 1

$$\text{RSA (\%) /\% Inhibition} = \left[\frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}}\right] \times 100$$

Table 1: Collected parts of the selected plants

Common name of the plant	Scientific name	Parts collected
Kaha Goraka	*Garcinia zeylanica*	leaves, bark, stem
Iriweriya	*Plectranthus zeylanicus*	leaves, stem, bark, shoots, roots
Girithilla	*Argyreia populifolia*	leaves, stem, bark, shoots, roots
Hora	*Dipterocarpus zeylanicus*	heartwood
Rath Goraka	*Garcinia quaesita*	leaves, bark, stem
Pupula	*Vernonia zeylanica*	leaves, stem, bar
Ruk	*Horsfieldia iryaghedhi*	bark
Gal Siyambala	*Dialium ovoideum*	leaves, bark
Kekuna	*Canarium zeylanicum*	bark

Results and discussion

DPPH Radical Scavenging Assay

The DPPH radical scavenging assay measures the electron donation ability of natural products using a very stable free radical DPPH.[1,6,9] This is a spectroscopic method based on the scavenging of the DPPH radical by the antioxidants. Highly stable free radical PPH has a dark purple colour in solution, which gets reduced due to the presence of radical scavengers.[10] The absorbance at 516 nm decreases when the DPPH radical is converted to 2,2-Diphenyl- 1-picrylhydrazine by antioxidants which can donate hydrogen radicals.[1] Therefore, the absorbance intensity at 516 nm decreases with the radical scavenging ability of the sample. The radical scavenging activities of the methanolic extracts of the plants were, *G. quaesita* (95±1%), *H. iryaghedhi* (94.9±0.2%), *G. zeylanica* (93.3±0.1%), *D. ovoideum* (92.9±0.4%), *D. zeylanicus* (91.1±1%), *V. zeylanica* (90±1%), *A. populifolia* (77±9%), *P.zeylanicus* (75.4±0.4%), *C. zeylanicum* (63±1%) and is represented as a bar chart in Figure 1. In order to determine the total phenolic activity of a sample by the Folin-Ciocalteu assay, a particular reference compound has to be used.[12] Phenolic compounds such as gallic acid, pyrogallol, and catechin are such commonly used reference compounds.[12] The standard curve plotted for the reference compound Pyrogallol, is shown in Figure 3. The absorbance values for plant extract samples were converted to the corresponding pyrogallol concentrations using this standard curve ($y = 0.9423x + 0.0009$, $R^2 = 0.9943$). Results were expressed in mg pyrogallol equivalents per 1 g of dried plant material.

In the presence of phenolic compounds the yellow coloured Folin-Ciocalteu reagent produces a blue colour. It is predicted that the colour change is due to the reduction of the phosphomolybdate-phosphotungstate complex forming a Mo (V) species.[1] Therefore, depending on the phenolic content of the sample, the absorbance intensity at 750 nm would vary. Although this method is

commonly used to measure the total phenolic content of a test sample, other reducing agents can also interfere with the test.[1]

According to the Folin-Ciocalteu assay the descending order of total phenolic contents of the plants was,

G. quaesita (293±3 mg (PGE)/g),
G. zeylanica (274.5±16.8 mg (PGE)/g),
D. ovoideum (192.9±11.4 mg (PGE)/g),
H. iryaghedhi (148.8±12.9 mg (PGE)/g), *V. zeylanica* (80.8±1.2 mg (PGE)/g),
D. zeylanicus (64.4±2.1 mg (PGE)/g),
A. populifolia (53.9±5.6 mg (PGE)/g),
C. zeylanicum (49.2±3.0 mg (PGE)/g),
P. zeylanicus (34.8±3.2 mg (PGE)/g).

Figure 4 shows the obtained total phenolic contents of the tested samples.

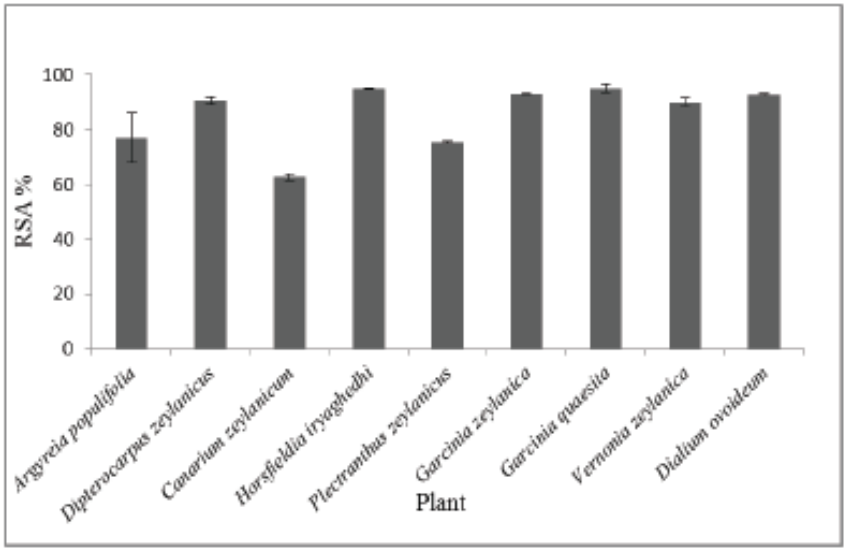

Figure 1: Radical scavenging activities of selected plants

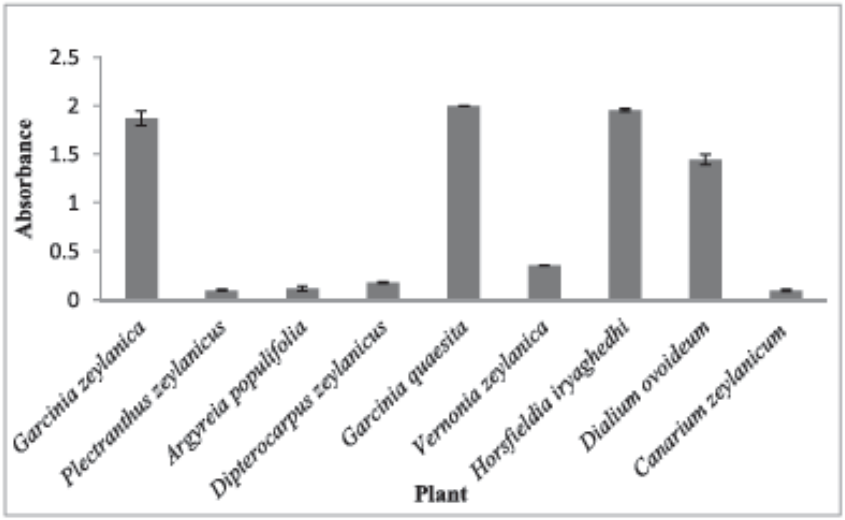

Figure 2: Reducing ability of the selected plant extracts

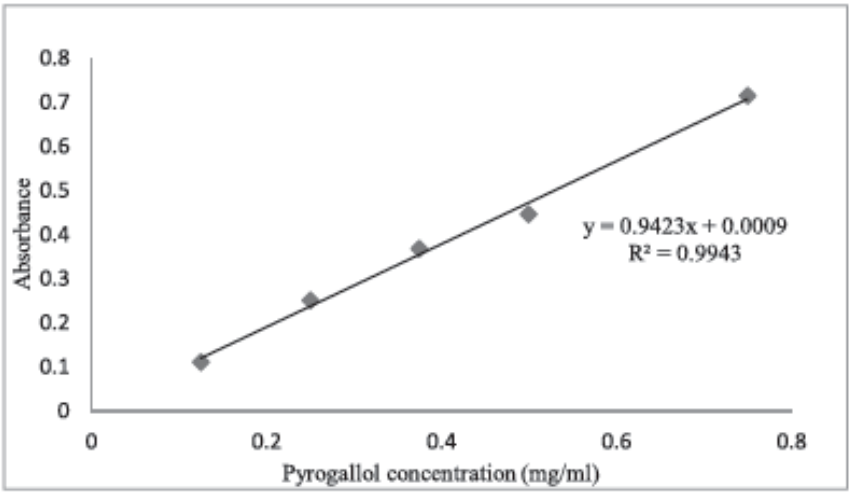

Figure 3: The standard curve of pyrogallol

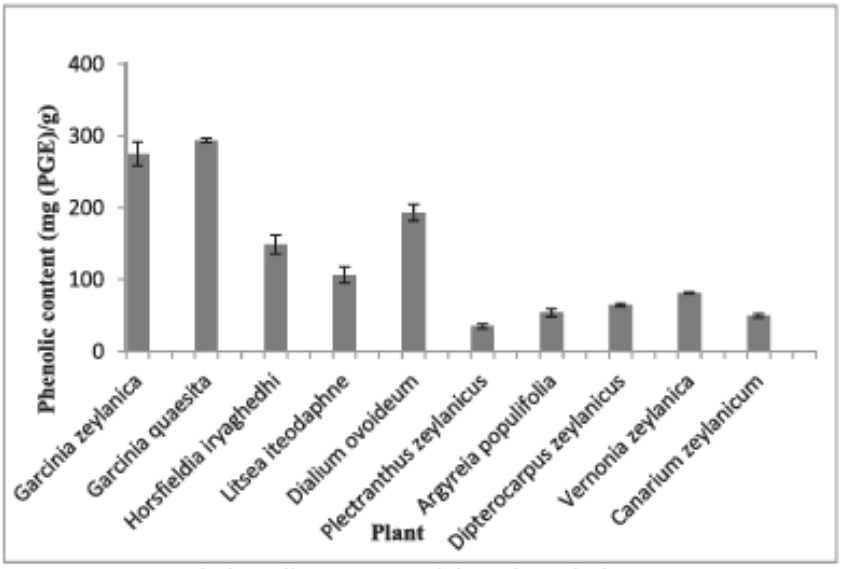

Figure 4: Total phenolic contents of the selected plant

In conclusion, this study shows that the Sri ankan endemic plants *Garcinia quaesita*, *Horsfieldia iryaghedhi, Garcinia zeylanica* and *Dialium ovoideum* have a high radical scavenging activity as well as a high reducing power and therefore show great promise to be developed as dietary supplements to counteract many diseases caused by radicals that are formed due to oxidative stress.

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