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Evaluation of antioxidant properties of 20 medicinal plant extracts traditionally used in Ayurvedic medicine in Sri Lanka

[1]*Attanayake A P & [1]Jayatilaka K A P W

[1]Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka-80000
E-mail: anoja715@yahoo.com

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A wide array of Sri Lankan medicinal plants has been used for the treatment of many chronic diseases such as diabetes mellitus, liver diseases and arthritis in Ayurvedic medicine. Antioxidants are reported to play central roles in reducing or preventing free radical damage in the above oxidative stress related diseases. The present study was conducted to evaluate the antioxidant activities of hot water extracts of 20 Sri Lankan medicinal plants. The total polyphenol content was determined by Folin-Ciocalteu method. The antioxidant activities were determined by three methods namely 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging activity, ferric reducing power (FRAP) and nitric oxide (NO) inhibition. Gallic acid and L-ascorbic acid were the reference compounds in expressing the polyphenol content and the antioxidant activities by the three assays, respectively. The total polyphenol content varied from 1.84 to 26.25 mgGAE /gm. The antioxidant activities ranged from IC_{50} of 19.48-245.68 µg/mL for DPPH radical scavenging, 103.35-484.60 µg/mL for NO inhibition and 1.14-26.06 µM for Ferric reducing antioxidant power (FRAP). A significant positive correlation was found between total polyphenol content and antioxidant activities indicating polyphenol compounds contribute significantly to the total antioxidant properties of medicinal plant extracts.

Keywords: DPPH, FRAP, IC_{50}, NO inhibition assay, Polyphenol content, Medicinal plant extracts

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Since 1990s, antioxidant research has expanded dramatically due to its potential benefits in disease prevention and health promotion. Oxidative stress caused by reactive oxygen species (ROS) results in an increased risk for many diseases such as inflammatory disease, cardiovascular disease, cancer, diabetes mellitus, liver disease, Alzheimer's disease and cataracts. Antioxidants may directly react with the reactive radicals to destroy them by accepting or donating electron(s) to eliminate the radical, or they may indirectly decrease the formation of free radicals. All human cells protect themselves by multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems against free radical damage. However, these protective mechanisms may not be enough to prevent severe or continued antioxidant stress[1]. Hence, certain amount of antioxidant is recommended for oxidative stress related diseases. Compounds especially from natural sources are capable of protecting against ROS mediated damage which may have potential applications in preventing or curing diseases[2].

*Corresponding author

Much of the wealth of a country resides in its inheritance of plants, whether the plants are endemic or natural[3]. There has been a wide array of medicinal plants used in the Ayurvedic treatments for many of the chronic diseases such as diabetes mellitus, liver diseases and arthritis. Indeed, antioxidants are reported to play a central role in reducing or preventing oxidative stress especially in the above diseases. However, complete *in vitro* screening on antioxidant activities of Sri Lankan medicinal plant extracts used in traditional medicine for the treatment of oxidative stress related diseases were not reported to date. Medicinal plants/parts selected for the investigation are listed with their medicinal use in Table 1. These plants are widely used in local Ayurvedic medicine for the treatment of oxidative stress related diseases such as diabetes mellitus, liver diseases and chronic rheumatism. Indeed, most of them are recommended as dietary adjuncts to the existing therapies[4]. Based on the traditional way of consumption, we found that it is appropriate to study the water-soluble antioxidant capacities of medicinal plant extracts.

The chemical approaches facilitate the study of antioxidants and their precise mechanisms of action[1]. Thus far, numerous studies on antioxidant properties of many plant species have been conducted using different assay methods. The general recommendation is to employ at least three *in vitro* methods due to the presence of a wide variety of oxidation systems[5].

The objectives of the study were to evaluate the total polyphenol content, *in vitro* antioxidant activities and to determine the relationship between polyphenol content and antioxidant activities of aqueous extracts of 20 medicinal plants traditionally used in Ayurvedic medicine in Sri Lanka.

Materials and methods

Plant material

A total of 20 samples, representing 20 Sri Lankan medicinal plant species from 14 families were collected during May-June from the Southern region of Sri Lanka (Table 1). The Botanical identities of all plants were determined by the descriptions given by Jayaweera[4] and confirmed by comparing with the authentic samples at the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. Voucher specimens have been deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.

Chemicals and reagents

All chemicals and solvents were of analytical grade and used without any purification.

Preparation of extracts

Selected plant parts were cut into small pieces, dried at 40°C until a constant weight was reached and coarsely ground. Powdered plant material (2.50 gm) was dissolved in 60.0 mL of distilled water and refluxed for two hours. The mixture was filtered and the final volume was adjusted to 50.0 mL. The concentration of the extract was 0.05 gm/mL. A concentration series of the extracts were prepared (1-500 µg/mL) for the DPPH and NO inhibition assays.

Determination of total polyphenol content

Total polyphenol content was measured by using Folin-Ciocalteu spectrophotometric method[6]. Quantification was done with respect to the standard curve of gallic acid. The results were expressed in gallic acid equivalents (GAE) mg GAE /gm of the dry weight.

DPPH Free radical scavenging activity

The total antioxidant activity was measured by the DPPH radical scavenging assay method[7,8]. The radical scavenging activity of plant extracts against stable DPPH radical (DPPH*) was determined. L-Ascorbic acid was used as the reference compound. The antioxidant activity is expressed in terms of IC_{50} (concentration of the extract / reference compound required to inhibit DPPH radical formation by 50%).

Ferric reducing antioxidant potential (FRAP assay)

The FRAP assay was performed according to the method of Benzie and Strain[9]. The assay was based on the reducing power of a compound (antioxidant). A potential antioxidant reduces the ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm.

Nitric oxide (NO) radical scavenging assay

Nitric oxide generated from sodium nitroprusside in an aqueous solution at physiological *p*H, interacts with the Griess reagent and the absorbance of the chromophore formed was measured using spectrophotometry[10].

Statistical analysis

Results were expressed as mean ± standard deviation of the three analytical triplicates. Correlation between polyphenol content and antioxidant activity was established by linear regression and correlation analysis. The level of significance was set as $p< 0.05$.

Results

The total polyphenol content of the 20 aqueous medicinal plant extracts were determined by regression analysis of gallic acid calibration curve. The total polyphenol content varied widely in a range of 1.84-26.25 mg GAE/gm of dry weight (Fig. 1). It was noticed that *Syzygium cumini* (L.) Skeels showed the highest total polyphenol content followed by *Osbeckia aspera* Blume, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. and *Nauclea orientalis* (L.) L. extracts.

All extracts and the standard compound (L-ascorbic acid) exhibited concentration-dependent radical scavenging activities in DPPH and NO inhibition assays as shown in Table 2. The radical scavenging activities were expressed in IC_{50} values in those two assays to obtain a more precise single

Table 1—Medicinal plants selected for the investigation

Plant name	Family	Part tested	Medicinal uses
Justicia adhatoda L. syn *Adhatoda vasica* Nees	Acanthaceae	leaves	chronic congestion of the liver
Alternanthera sessilis (L.) R.Br. ex DC.	Amaranthaceae	aerial arts	chronic congestion of the liver
Aerva lanata (L.) Juss.	Amaranthaceae	whole plant	liver diseases, diabetes mellitus
Hemidesmus indicus (L.) R. Br. ex Schult.	Asclepidaceae	aerial parts	inflammation of urinary passages, chronic rheumatism
Dregea volubilis (L.f.) Benth. ex Hook.f.	Asclepidaceae	aerial parts	diabetes mellitus
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	Combretaceae	bark	liver disease
Benincasa hispida (Thunb.) Cogn.	Cucurbitaceae	fruit	diabetes mellitus
Swertia chirata Buch.-Ham. ex Wall. syn *Agathotes chirayta* D.Don	Gentianaceae	aerial part	liver disease
Chrysopogon zizanioides (L.) Roberty syn *Vetiveria zizanioides* (L.) Nash	Gramineae	root	chronic congestion of the liver
Plectranthus hadiensis (Forssk.) Schweinf. ex Sprenger syn *Plectranthus zeylanicus* Benth.	Labiatae	aerial part	chronic congestion of the liver
Osbeckia aspera Blume	Melastomaceae	leaves	liver disease
Azadirachta indica A.Juss.	Meliaceae	leaves	diabetes mellitus
Coscinium fenestratum (Goetgh.) Colebr.	Menispermaceae	wood	diabetes mellitus
Syzygium cumini (L.) Skeels	Myrtaceae	bark	diabetes mellitus
Nauclea orientalis (L.) L.	Rubiaceae	bark	liver disease
Oldenlandia biflora L.	Rubiaceae	whole part	liver disease
Pavetta indica L.	Rubiaceae	leaves	chronic rheumatism
Nigella sativa L.	Ranunculaceae	seeds	liver disease, diabetes mellitus
Withania somnifera (L.) Dunal	Solanaceae	root	diabetes mellitus, liver disease
Coriandrum sativum L.	Umbelliferae	seeds	liver disease

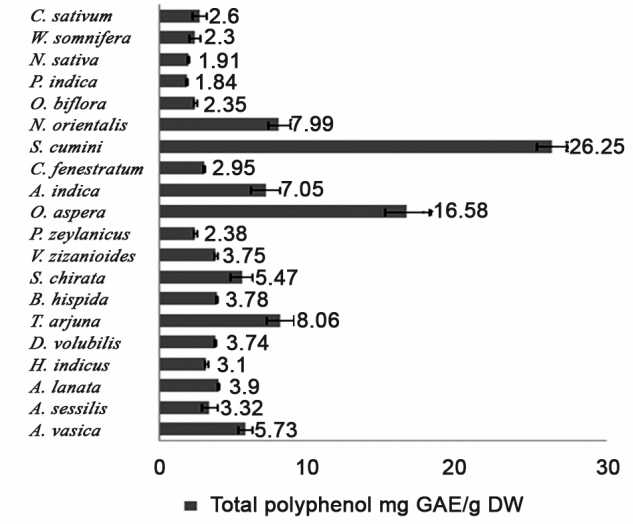

Fig 1—Total polyphenol content of plant extracts. Data are expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. All values were the mean of three measurements and expressed as mean ± SD.

value over a range of concentrations of plant extracts. The IC_{50} values in DPPH and NO radical inhibition assays were less than 245.68 and 484.60 μg/mL, respectively.

As shown in Table 2, the ferric reducing power of extracts were between 1.14-26.06 μM at the concentration of 0.05 gm/mL and followed the in the decreasing order of *Syzygium cumini* (L.) Skeels, *Osbeckia aspera* Blume, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., *Nauclea orientalis* (L.) L. and *Oldenlandia biflora* L. The extract of *Withania somnifera* (L.) Dunal has the lowest ferric reducing power among the selected plant extracts.

The linear regression and correlation analysis were done to establish the correlation between total polyphenol content and antioxidant activities of medicinal plant extracts and to evaluate the suitability and reliability of the three *in vitro* assay methods. The correlation coefficients (*R*) and coefficients of

Table 2—Total polyphenol content and antioxidant activities of 20 Sri Lankan medicinal plant extracts[a]

Plant name/ reference compound	Total antioxidant activities		
	IC_{50} in DPPH assay µg/mL [b]	FRAP assay µM [c]	IC_{50} in NO assay µg/mL [d]
Justicia adhatoda L. syn *Adhatoda vasica* Nees	151.57±1.56	4.35±0.23	241.70±3.45
Alternanthera sessilis (L.) R.Br. ex DC.	165.39±2.03	1.21±0.02	432.64±3.67
Aerva lanata (L.) Juss.	139.06±2.00	2.12±0.07	346.87±3.02
Hemidesmus indicus (L.) R. Br. ex Schult.	142.98±1.78	3.18±0.47	346.15±2.75
Dregea volubilis (L.f.) Benth. ex Hook.f.	139.52±1.63	1.63±0.89	460.21±4.05
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	130.06±1.45	9.36±0.34	168.68±2.00
Benincasa hispida (Thunb.) Cogn.	157.80±1.58	2.23±0.08	475.26±3.56
Swertia chirata Buch.-Ham. ex Wall. syn *Agathotes chirayta* D.Don	150.02±1.50	4.14±0.24	411.14±4.67
Chrysopogon zizanioides (L.) Roberty syn *Vetiveria zizanioides* (L.) Nash	189.28±1.84	2.87±0.34	384.96±3.00
Plectranthus hadiensis (Forssk.) Schweinf. ex Sprenger syn *Plectranthus zeylanicus* Benth.	182.23±2.05	1.28±0.10	432.33±2.45
Osbeckia aspera Blume	113.21±1.23	15.84±0.90	165.34±3.33
Azadirachta indica A.Juss.	133.76±1.58	4.82±0.23	250.52±2.34
Coscinium fenestratum (Goetgh.) Colebr.	121.30±1.45	2.74±0.16	481.38±3.30
Syzygium cumini (L.) Skeels	19.48±1.01	26.06±0.10	103.35±1.23
Nauclea orientalis (L.) L.	122.96±.56	7.87±0.11	188.85±2.56
Oldenlandia biflora L.	131.87±1.90	4.75±0.23	471.33±3.56
Pavetta indica L.	144.21±1.45	1.86±0.67	432.02±4.09
Nigella sativa L.	158.28±2.00	1.25±0.45	381.85±2.45
Withania somnifera (L.) Dunal	245.68±2.01	1.14±0.03	484.60±3.56
Coriandrum sativum L.	155.94±1.23	1.85±0.20	434.14±3.89
L-Ascorbic acid	4.52±0.89	2.00±0.00	28.59±0.80

[a] All values are the mean of three measurements and expressed as mean ± SD.
[b] Radical scavenging activity by DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) assay is expressed in IC_{50} (Concentration of the extract / solution required to inhibit DPPH radical formation by 50%)
[c] FRAP(ferric reducing power). Data are expressed as µM.
[d] Radical scavenging activity by NO (Nitric oxide) inhibition assay is expressed in IC_{50} (Concentration of the extract / solution required to inhibit NO radical formation by 50%)
Correlations (R and R^2) between total polyphenol content (TPC) and $1/IC_{50}$ (Concentration of the extract required to inhibit DPPH radical formation by 50%) in DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate), FRAP(ferric reducing power) value and IC_{50} (Concentration of the extract required to inhibit nitric oxide radical formation by 50%) in NO (nitric oxide) inhibition assay of 20 aqueous Sri Lankan medicinal plant extracts. [a]R, correlation coefficient. R^2, coefficient of determination. The values in parenthesis represent R^2 values.* Significance level at $p < 0.05$

determinations ($R2$) are given in Table 3. The results firmly establish a strong correlation between the total polyphenol content and antioxidant activity (Figs. 2-4). In our study, the total polyphenol content and antioxidant activities were positively correlated as R=0.982, 0.942, 0.851 in FRAP, NO and DPPH assays, respectively.

Discussion

The Folin–Ciocalteu procedure has been proposed as a rapid method to quantify the total polyphenol content in medicinal plant extracts[11]. Polyphenol compounds of plant origin are viewed as promising neutraceuticals, especially from a health point of view. The antioxidant effect of polyphenol compounds is mainly due to their redox properties and as a result of various possible mechanisms including free radical scavenging activity, metal chelating activity and/or singlet oxygen-quenching capacity. In addition, they are known to play an important role in stabilizing lipid peroxidation and inhibit various types of oxidizing enzymes[12].

In this study *in vitro* antioxidant activities were evaluated by three methods; DPPH, FRAP and NO assays. The IC_{50} values of the extracts were calculated for DPPH and NO inhibition assays to compare the

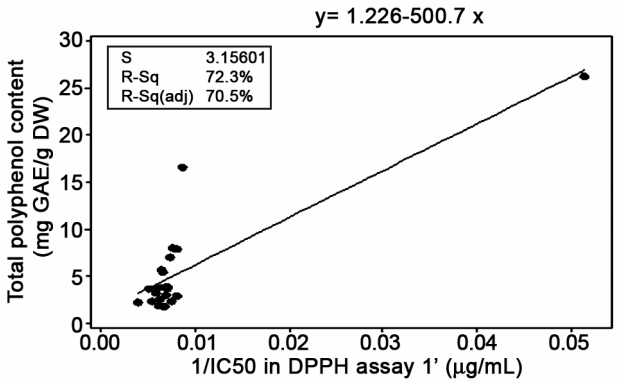

Fig 2—Relationship between total polyphenol content and DPPH radical scavenging assay of 20 medicinal plant extracts. Relationship between total polyphenol content and $1/IC_{50}$. Total polyphenol content is expressed as milligrams of gallic acid equivalents GAE per gram of dry weight.

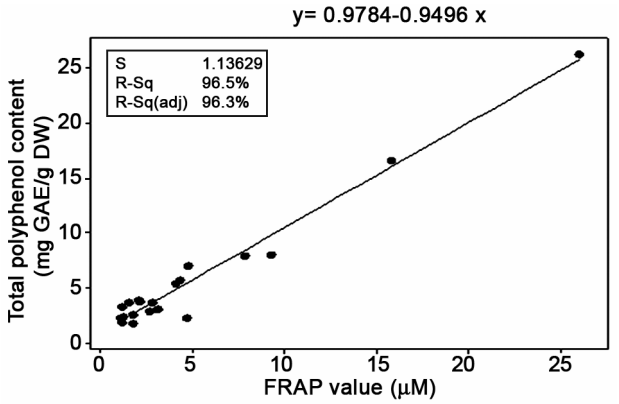

Fig 3—Relationship between total polyphenol content and FRAP value of 20 medicinal plants extracts. FRAP value is given as µM. Total polyphenol content is expressed as milligrams of gallic acid equivalents GAE per gram of dry weight.

antioxidant activities at different concentrations as described by many authors[13,14]. These results are in accordance with the findings of several authors[15]. In this study, the highest amount of polyphenol content has led to highest radical scavenging activity (lowest IC_{50}) as shown in the extract of *Syzygium cumini* (L.) Skeels in both assays. In contrast, the highest IC_{50} was found in the extract of *Withania somnifera* (L.) Dunal in DPPH and NO inhibition assay. The radical scavenging activity in DPPH assay was in the decreasing order of *Osbeckia aspera* Blume, *Coscinium fenestratum* (Goetgh.) Colebr., *Nauclea orientalis* (L.) L., *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., followed by *Osbeckia aspera* Blume, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., *Nauclea orientalis* (L.) L. and *Azadirachta indica* A.Juss. in the NO inhibition assay. However,

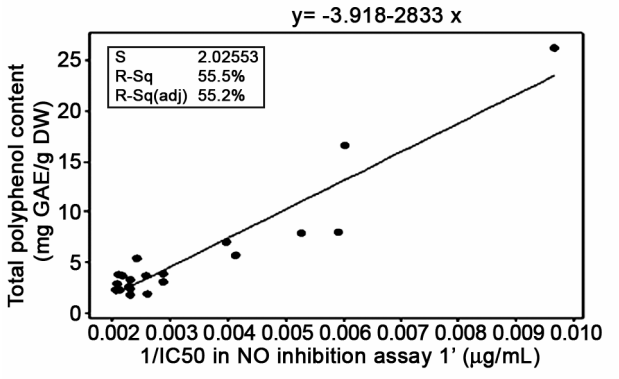

Fig 4—Relationship between total polyphenol content and NO radical scavenging activities of 20 medicinal plants extracts Relationship between total polyphenol content and $1/IC_{50}$. Total polyphenol content is expressed as milligrams of gallic acid equivalents GAE per gram of dry weight.

Table 3—Correlation between polyphenol content and antioxidant activities of medicinal plant extracts

R and R^{2a}	DPPH	FRAP	NO
FRAP	0.849 *(0.721)		
NO	0.788 *(0.621)	0.950 *(0.902)	
TPC	0.851 *(0.723)	0.982 *(0.965)	0.942 *(0.888)

the IC_{50} of standard compound (L-ascorbic acid) as a powerful antioxidant was more pronounced in DPPH and NO radical scavenging assays and the values are comparable with the previous studies[16]. The ferric reducing capacity of a compound serves as a significant indicator of its potential antioxidant activity[17]. The three assays used in this study to assess the antioxidant capacity are spectrophotometric methods. However, it is not surprising to find differences in the antioxidant activity measurements among the assays, as each has a different mechanism of action and/or different reaction conditions. DPPH assay is based on the ability of DPPH, a stable free radical to decolorize in the presence of antioxidants. This is reported to be a direct and a reliable method for the determination of radical scavenging activity where the structure of electron donor (e.g. plant extract) is not known. DPPH assay method can afford data on reduction potential of the sample and hence can be helpful in comparing the reduction potential of unknown compounds[11]. A key mediator released by activated macrophages that has been implicated in cellular toxicity is nitric oxide. A large number of studies have shown that increased amounts of highly reactive nitrogen intermediates are produced during tissue injury associated with inflammation. It has

been pointed out that modulating nitric oxide production can modify tissue injury[18]. Nitric oxide radicals combine with superoxide radical and forms peroxynitrite ($ONOO^-$) which is cytotoxic. Due to lack of endogenous enzymes responsible for $ONOO^-$ inactivation, development of specific $ONOO^-$ scavengers or NO scavengers is considered important[19].

FRAP assay has many advantages over radical scavenging assays such as excellent reproducibility, linearity over a wide range and high sensitivity. In contrast, the FRAP assay measures the reducing capability by increased sample absorbance and the assay may not complete even several hours after the reaction starts, such that a single end point of the reaction cannot be determined[7].

In our study the total polyphenol content and antioxidant activities were positively correlated. It is pertinent to mention that earlier reports have demonstrated significant correlations between polyphenol contents and antioxidant power[20]. In the present study, all *R* values were positive at the $p < 0.05$ significance level. This indicates that the three antioxidant assays are suitable and reliable for assessing the total antioxidant potentials of plant extracts, although there were some samples with high polyphenol content with differences in antioxidant capacities between assay methods.

Conclusion

The study demonstrates for the first time that the selected 20 medicinal plant extracts possess antioxidant activities with high content of polyphenol compounds. The extracts of *Syzygium cumini* (L.) Skeels, *Osbeckia aspera* Blume, *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. and *Nauclea orientalis* (L.) L. are with excellent antioxidative potentials. The highly significant correlations obtained in this study suggest that polyphenol compounds contribute significantly to the total antioxidant capacities of medicinal plant extracts. The study suggests that the selected 20 medicinal plant extracts reduce the oxidative damage induced by radicals and scrutinize the therapeutic uses in traditional Ayurvedic medicine.

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