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Antioxidant and free radical scavenging capacity of extensively used medicinal plants in Sri Lanka

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Abstract

The burden of chronic diseases is rapidly increasing worldwide. Diet and nutrition are important factors in the promotion and maintenance of good health throughout the entire life course. Physiological and biochemical alterations in the human body may result in overproduction of free radicals leading to oxidative damage to biomolecules (e.g. lipids, proteins, DNA). Use of medicinal plant based products has increased recently because of their exerted beneficial properties such as antioxidant, anticancer, hypoglycaemic and hypolipidaemic activities. The present study was designed to assess the *in vitro* antioxidant activity and free radical scavenging capacity of ten medicinal plants which are extensively used in the Ayurvedic treatment systems in Sri Lanka. Water extracts were prepared and evaluated for their free-radical scavenging capacity and antioxidant activity using a number of chemical assays; DPPH, ABTS and FRAP. The total Phenolic (TPC) and Total Flavonoid Content (TFC) were also assessed. The TPC and TFC values of the extracts varied from $295.94 \pm 3.65 - 5.22 \pm 0.08$ (mg Gallic Acid Equivalent (GAE)/g dry weight) and $115.01 \pm 1.69 - 0.97 \pm 0.002$ (mg Catechin Equivalent (CE)/g dry weight) respectively. The DPPH and ABTS radical scavenging activities were higher for the Nelli (*Phyllanthus emblica*) extract while the least activity was observed in Venivel (*Coscinium fenestratum*) extract. The FRAP activity of the extracts was well proved with the DPPH and ABTS radical scavenging activities. A positive, significant linear relationship between antioxidant activity and TPC and TFC content showed that phenolic compounds and flavonoids were the dominant antioxidant components in the medicinal herbs studied.

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2. Introduction

The burden of chronic diseases is rapidly increasing worldwide. It has been calculated that, in 2001, chronic diseases contributed approximately 60% of the 56.5 million total reported deaths in the world and approximately 46% of the global burden of disease¹. Reactive free radicals, including superoxide, hydroxyl radical, and peroxy radical, generally result in degradation of protein, lipid peroxidation, and oxidation of DNA, which have been considered to be linked with many chronic diseases, such as diabetes, cancers, and atherosclerosis. Several studies have demonstrated the potential role of fruits and vegetable in promoting health and preventing diseases. Diet has been known for many years to play a key role as a risk factor for chronic diseases. Plant phenols have been of interest for scientists for decades, especially owing to their health benefits. Herbs have been used as food and for medicinal purposes for centuries. During the past two decades there has been a tremendous resurgence in the interest and use of medicinal plant products and an intense interest in “nutraceuticals” or “functional foods” in which phytochemicals can have long-term health promoting or medicinal properties. In different herbs, a wide variety of active phytochemicals, including the flavonoids, terpenoids, alkaloids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides have been identified. For centuries Sri Lankans have been using several native herbs for food and for medicinal purposes. Therefore, the present work aims at evaluating the *in vitro* antioxidant activity of ten medicinal herbs commonly used in the Ayurvedic system of Sri Lanka. The latest scientific evidence on the nature and strength of the links between diet and chronic diseases is examined and discussed in the recent study.

3. Methodology

Water extracts of the herbs; Belimal (*Aegle marmelos*), Iramusu (*Hamidesmus indicus*), Ranawara (*Cassia auriculata*), Walkottamalli (*Scoparia dulcis*), Nelli (*Phyllanthus emblica*), Rasakinda (*Tinospora cordifolia*), Polpala (*Aerva lanata*), Babila (*Sida rhombifolia*), Beligeta (*Aegle marmelos*) and Venivel (*Coscinium fenestratum*), were prepared by boiling 20 g of the herb in 200 ml for 5 min and the extracts were stored under -20 °C till further analysis.

2.1. Total Phenolic Content

The total phenolic content (TPC) of the extracts was determined calorimetrically with some modifications². The total phenolic content was determined by comparison with the standard calibration curve of Gallic acid. Results were presented as milligrams of Gallic acid equivalents (mg of GAE) per gram dry weight (g DW).

2.2. Total Flavonoid Content

The assay was performed by the aluminum chloride assay through colorimetric, following the procedure described by² and³

3.3 .Antioxidant Capacity

2.3.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The DPPH assay was performed according to the method described by⁴ with modifications. The sample concentration providing 50% inhibition (IC₅₀) was calculated from the graph of inhibition percentage against sample concentration.

2.3.2. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Activity

The total antioxidant capacity of the extracts was determined using ABTS radical. Absorbance was read over 6 minutes at 1 minute interval at 734 nm. The radical scavenging activity after lapse of 6 minute was calculated as percentage of ABTS discolouratio⁵ ABTS Radical Scavenging Activity was also determined by comparison with

the standard calibration curve of Trolox. Results were presented as mM of Trolox equivalents (mM of TE) per gram dry weight (g DW).

2.3.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The ability to reduce ferric ions was measured using the method described by,⁶ with some modifications. Fresh working solutions of FeSO₄ were used for the standard curve. The antioxidant capacity based on the ability to reduce ferric ions of sample was calculated from the linear calibration curve and expressed as mM FeSO₄ equivalents per gram of sample.

2.4. Statistical analysis

Three replications were used to obtain average values and standard deviations for all tests. All results were analyzed using SAS version 9.1 statistical software (SAS Institute Inc. NC. USA). CRD model was used to evaluate the differences in antioxidant properties. Probability (P) ≤ 0.05 indicates significance.

4. Result and Discussion

The TPC and TFC values of the extracts varied from 295.94±3.65 – 5.22±0.08 (mg Gallic Acid Equivalent (GAE)/g dry weight) and 115.01±1.69 – 0.97±0.002 (mg Catechin Equivalent (CE)/g dry weight) respectively. According to the statistical analysis (P≤0.05) the TPC and TFC were markedly higher in the Nelli extract (Table 1). In the DPPH Free radical scavenging activity, water extracts of the herbs were evaluated for their free radical scavenging activity. Table 1 indicates the IC₅₀ value for each tested sample. The IC₅₀ value of Nelli was observed to be very low (0.02±0.00 mg/ml) indicating highest antioxidant activity according to the statistical analysis (P≤0.05) compared to other tested samples. The IC₅₀ values for other samples ranged from 0.21±0.01 – 7.89±0.20 mg/ml. The least activity was recorded in the Venivel extract.

Table 1. Total phenolic content (TPC) and antioxidant activity of the herb extracts

| Plant | TPC (mg GAE/g) | TFC (mg CE/g) | ABTS (mM TE/g) | DPPH (IC ₅₀) (mg/ml) | FRAP (mM Fe ²⁺ /g) |
|---------------|--------------------------|--------------------------|----------------------------|----------------------------------|-------------------------------|
| Belimal | 44.27±0.03 ^d | 24.03±0.55 ^b | 130.72±1.73 ^c | 0.46±0.00 ^g | 413.21±10.22 ^b |
| Beligeta | 47.55±1.29 ^c | 16.81±1.20 ^d | 106.43±0.09 ^d | 0.97±0.04 ^f | 192.01±8.34 ^{cd} |
| Iramusu | 8.57±0.24 ^g | 3.24±0.170 ^g | 19.65±0.05 ^{gh} | 2.79±0.09 ^d | 65.31±1.49 ^e |
| Nelli | 295.94±3.60 ^a | 115.01±1.69 ^a | 1106.96±15.00 ^a | 0.02±0.00 ^h | 5355.89±47.82 ^a |
| Rasakinda | 5.22±0.06 ^h | 0.97±0.00 ^h | 11.00±0.14 ^{ih} | 5.64±0.14 ^b | 41.92±0.98 ^e |
| Babila | 12.96±0.38 ^f | 5.74±0.03 ^e | 27.86±0.17 ^f | 2.00±0.07 ^e | 95.55±3.35 ^{de} |
| Polpala | 10.33±0.32 ^g | 6.85±0.3 ^{ef} | 18.16±0.13 ^{gh} | 5.25±0.14 ^c | 26.19±0.51 ^e |
| Ranawara | 51.87±0.43 ^b | 19.60±0.34 ^c | 194.61±3.20 ^b | 0.21±0.01 ^h | 478.63±12.13 ^b |
| Venivel | 5.22±0.08 ^h | 2.78±0.08 ^g | 9.19±0.14 ⁱ | 7.89±0.20 ^a | 35.11±1.71 ^e |
| Walkottamalli | 29.71±0.76 ^e | 7.73±0.04 ^e | 71.11±0.25 ^e | 1.02±0.01 ^f | 228.24±6.70 ^c |

Means followed by the same letter within each column are not significantly different at P≤0.05, according to the least significant difference test.

The ABTS free radical scavenging activity of the extracts well correlated with the DPPH results. According to the statistical analysis (P≤0.05) Nelli extract had the highest total antioxidant capacity of 1106.96±15.00 mM Trolox Equivalent (TE)/ g dry weight while the Venivel extract had the lowest activity of 9.19±0.14 mM Trolox Equivalent (TE)/ g dry weight (P≤0.05).

The ferric reducing ability of the extracts was in the range of 26.19±0.51±0.11 – 5355.89±47.82 mM of Fe (II)/g dry weight (Table 1). According to the statistical analysis (P≤0.05) Nelli extract had the highest ability to reduce Fe

(II) followed by Ranawara and Belimal. The Polpala₂ extract had the least activity in reducing the ferric ions ($P \leq 0.05$).

5. Conclusion

Results from the present study showed that water extracts of the ten herbs tested have potential antioxidant activity and are capable of scavenging reactive oxygen species. Especially, the Nelli₂ extract exhibited significantly higher antioxidant activity than the other samples which may be attributed to the higher phenolic content since a linear relation was observed between the TPC and the antioxidant parameters. A more detailed study on the bioactive compound in these plant extracts that contribute to these biological activities as well as their possible mechanism of action are therefore suggested.

6. References

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