Evaluation of Antioxidant Activity of Five Medicinal Plants in Sri Lanka.

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Submission Date: 16-2-2014
Accepted Date: 24-3-2014

ABSTRACT

This study investigated on the antioxidant properties of five medicinal plants used in Sri Lanka, namely *Solanum nigrum*, *Amaranthus spinosus*, *Elephantopus scaber*, *Amorphophallus campanulatus* and *Canna indica*. The cold methanol plant extracts were screened for the antioxidant activity evaluating their 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in scavenging ability. The total ascorbic acid content of the extracts was also evaluated. The IC₅₀ values of the extracts revealed that *Solanum nigrum* had the best DPPH scavenging activity with a value of 37.63 ± 0.11µg/ml and was better than that of the standard ascorbic acid. *Amorphophallus campanulatus* extract gave the highest ascorbic acid content of 143.03 ± 1.97 mg per 100 g of the extract. All five plants extract showed DPPH scavenging activity in the order of *Solanum nigrum* > *Elephantopus scaber* > *Amorphophallus campanulatus* > *Canna indica*. The plant extracts did not show a direct correlation between the ascorbic acid content to the DPPH scavenging activity. These experimental results reveals that these extracts can be utilized in future as therapeutic agent against free radical induced oxidative stress.

Keywords: Antioxidant activities, Ascorbic acid, DPPH, Medicinal Plants

INTRODUCTION

The history of use of plants in traditional medicine in Sri Lanka dates back into 4th century BC. Two of such medical systems as Ayurveda and Deshiya Chikitsa use mainly plant and herbal preparations for the treatment of diseases. There are over 2000 plant species used for medicinal purposes and many of these plants are endemic to Sri Lanka. The uses of such medicinal plants are documented and manuscripts can be found among Ayurveda doctors.[¹] Many previous literature and practices reveal that plant material has been used as agents in curing and preventing many diseases as cancer, aging, skin diseases, cardiovascular disease, immune deficiency disease etc. due to their antioxidant properties.[², ³]

Today, many medicinal plants in most countries are extensively investigated in search of novel drugs with antioxidants, antitumor, anti-mutagenic and antimicrobial activities. Many pharmaceutical industries are also involved in harnessing medicinally important natural products from plants. Around 60% of antitumor drugs and anti-infective drugs that are available in the market today are of the natural origin.[⁴] In the recent past the interest towards searching for antioxidant from natural products has increased due to potent and cost-effectiveness of the antioxidants from various plant sources.[⁵, ⁶] Furthermore use of antioxidant in prevention and treatment of cancer has provided many clinical benefits and therefore extensively used in inhibiting, or delaying carcinogenesis.[⁷, ⁸] It is also reported that many undesirable health effects are experienced from the use of existing synthetic antioxidants in both food and drugs. With the latest trend many food technologists add crude plant extracts into many food product with the intention of increasing the nutrient values, taste and as antioxidants.

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DOI: 10.5530/pj.2014.3.8
Antioxidants have the capability of inhibiting radical reactions that lead to undesirable conditions both in human and in food products. Free radicals and oxygen species such as, hydroxyl radicals, superoxides and other singlet oxygen are generated in the human body under physiological conditions. However all cells are equipped with enzymes as superoxide dismutase glutathione peroxidase and catalase as a natural defense system against the oxidative damage that could be caused by reactive radical and oxygen species under normal conditions. Excessive generation of such as reactive species are believed to be formed under disease conditions as cardiovascular disease, aging, and neurodegenerative diseases Alzheimer’s disease, mutations and cancers.[9]

The plants exhibit their antioxidant activities due to naturally occurring compound as ascorbic acid, tocopherols, and polyphenols.[10] These antioxidants have been investigated and are used to protect the human body and food products from oxidative damage by inhibiting lipid peroxidation, scavenging free radicals and active oxygen species and chelating heavy metal ions.[11, 12] The addition of synthetic antioxidants, such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and tertiary butylhydroquinone has been widely used industrially. However, the uses of these synthetic antioxidants have been questioned due to their potential health risks and toxicity.[13]

The use of natural antioxidants for treating diseases and as food additives has better consumer acceptability and a trend over the use of the available synthetic products. Many research groups therefore have taken the responsibility of screening and quantification of the antioxidant activities of the medicinal plant.[14]

Therefore in this study we determined the in vitro antioxidant activities of five extracts of ayurvedic plant materials namely, *Solanum nigrum, Amaranthus spinosus, Elephantopus scaber, Amorphophallus campanulatus* and *Canna indica*, used in the Sri Lanka for many different medicinal purposes (Table 1). We determined their free radical scavenging activity against 1, 1-diphenyl-2-picyrylhydrazyl (DPPH) and the total ascorbic acid content. It is important that this information on the antioxidant properties is available prior to incorporating them into food products and be used as drug for controlling diseases.

**MATERIALS AND METHODS**

**Apparatus and materials**

Spectrophotometer, 2, 2-diphenil-1-picyrylhydrazyl (DPPH), Na₂CO₃, KIO₃, NaS₂O₃, 5H₂O, H₂SO₄, Soluble starch, Ascorbic acid, KI, NaHCO₃ (all chemicals used were in analytical grade or HPLC grade)

**Plant Material:** All plant materials were collected from the vicinity of University of Peradeniya, Peradeniya, Sri Lanka. Authenticity of the plant species was validated by the specific morphological and anatomical features from the authentic specimens available at the Sri Lanka Royal Botanical Gardens Peradeniya). The plant materials were air-dried at room temperature (26°C) for 2 weeks and thereafter grounded into a uniform powder for extraction.

**Extraction:** Methanol extracts were prepared by soaking 100 g of the dry powdered plant materials in 1L of methanol at room temperature for 48 h. The filtered extracts were concentrated under reduce pressure maintaining the temperature under 40°C.

**DPPH Assay:** The free radical scavenging ability of the extracts were determined using 2,2-diphenil-1-picyrylhydrazyl (DPPH) radicals as describe in Braça, et. al., 2002.[14] Ethanolic DPPH solution (0.05 mM) was freshly prepared and kept in the dark at 4°C until use. DPPH solution (300µl of 0.05mM) was added to 40µl of plant extract at different concentrations ranging from,12.5µg/ml to 100 µg/ml or into 40 µl of DI water (control). A volume of 2.7ml of Ethanol (96%) was added to the reaction mixture making the total volume to be 3.00 ml and was mixed vigorously. The mixture was left to stand for 30 min and the absorbance at 517 nm was measured. All experiments were performed in triplicate. The radical scavenging activities of the tested samples were expressed as percentage of inhibition was calculated according to the following equation.

Percent (%) Inhibition of DPPH Activity = [(A_control – A_sample)/A_control] × 100

The concentration of sample required for 50% inhibition was determined using linear regression analysis of the plot of extract concentration vs % inhibition and represented as IC₅₀ of the extract. Lower IC₅₀ value it indicates a greater antioxidant activity.

**Determination of Total Ascorbic acid content:**

Ascorbic acid content was determined by the iodometric (redox) titration method. Fresh and dried plant material (10g) was added to 75ml of CO₂ free DI water and filtered into a 100 ml volumetric flask and adjusted the volume up to 100.00 ml using distilled water. A volume of 20 ml of 0.01 M KIO₃, 1 g of KI, 0.1 g of NaHCO₃ and 10 ml of 0.2 MH₂SO₄ were added into the titration flask and thereafter 10ml of the above plant extract was added.
The mixture was titrated against 0.07 M Na$_2$S$_2$O$_3$ solution. Each extract was titrated in triplicates.

**RESULTS**

**DPPH Assay:** The absorbance values at 517 nm, for the mixture of the extract with the DDPH showed a decrease with the increase in the concentrations of plant extract. These values were used to calculate the percentage of inhibition which showed an increase in the percentage inhibition with the increase in the concentration of plant extract (Figure 1). Using the plot, the IC$_{50}$ value of each plant extract was evaluated. The IC$_{50}$ of the plant extracts of *Canna indica*, *Amorphophallus campanulatus*, *Elephantopus scaber*, *Amaranthus spinosus* and *Solanum nigrum* were, 601.76 ± 2.31µg/ml, 136.39 ± 0.32µg/ml, 69.15 ± 0.08µg/ml, 56.51 ± 0.74µg/ml and 37.63 ± 0.11µg/ml respectively. The IC$_{50}$ of the standard ascorbic acid was 40.71 ± 0.13µg/ml (Table 2; Figure 1). The plant extract

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Family</th>
<th>Common Name</th>
<th>Plant Part Used</th>
<th>Medicinal Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum nigrum</td>
<td>solanaceae</td>
<td>Kalukanweriya* (Sinhala) Nightshade (English)</td>
<td>leaf</td>
<td>For gouty joints and rheumatism, piles, gonorrhea, dropsy and enlargements of the liver and spleen, sore eyes and various skin diseases treatment of malaria, black – water fever and dysentery (Rhodesia); for erysipelas (Mexico); diabetes (Philippines) fever, diarrhea, eye diseases and hydrophobia (Bengal); a substitute for raisins (Africa)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fruit</td>
<td></td>
<td>abdominal pain and inflammation of the bladder (Mauritius); headaches, ulcers, wounds and as a diuretic and emetic (Europe); antispasmodic, diaphoretic, emollient and sedative (Italians); as a vegetable (Africa)</td>
</tr>
<tr>
<td>Amaranthus spinosus</td>
<td>amranthaceae</td>
<td>Katuthampala (Sinhala) Prickly Amaranth –English</td>
<td>plant</td>
<td>Sudorific, febrifuge and eruptive fevers, piles (Ghana); a sudorific, febrifuge and galactagogue (Philippine); as a diuretic (Malaya &amp; Mauritius)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leaves</td>
<td></td>
<td>Often eaten as a pot herb good emollient &amp; lactagogue properties for colic, locally for eczema.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>root</td>
<td></td>
<td>gonorrhea and mild diuretic and demulcent action</td>
</tr>
<tr>
<td>Elephantopus scaber</td>
<td>asteraceae</td>
<td>Eth adi(Sinhala) Elephant’s foot (English)</td>
<td>root</td>
<td>for urinary discharges, diarrhea, dysentery, dysuria and as a cardiac tonic (India); for cough (Malaya)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plant</td>
<td></td>
<td>anthelmintic for roundworms and a decoction, decoction for increasing the discharge of urine (India, China); diuretic and febrifuge (Madagascar); as a tonic, diaphoretic and emmenagogue and given for dyspepsia (West Indies); decoction as a diuretic, febrifuge and emollient in the (Philippine Island)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leaves</td>
<td></td>
<td>septic nails and wounds caused by bites of wild animals (Sri Lanka)</td>
</tr>
<tr>
<td>Amorphophallus campanulatus</td>
<td>araceae</td>
<td>Kidaran (Sinhala)</td>
<td>Corm</td>
<td>tonic, stomachic, antibacterial, antifungal, appetizer and cytotoxic activity, externally to relieve pain in acute rheumatism, eaten during periods of food scarcity, piles, acute dyspepsia, abdominal colic, elephantiasis, skin and blood diseases, fistula, glandular swelling in the neck, urinary disease and dropsy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>elephant foot yam (English)</td>
<td>Roots</td>
<td>for boils and hemorrhoids, and ophthalmia, abdominal pains, tumors, spleen enlargement, asthma and rheumatism</td>
</tr>
<tr>
<td></td>
<td></td>
<td>seed</td>
<td>tooth – ache</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>-tooth – ache</td>
<td>Tubs</td>
<td>hemorrhoids.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>diuretic, diaphoretic &amp; demulcent. a food</td>
<td></td>
</tr>
<tr>
<td>Canna indica</td>
<td>cannacea</td>
<td>Buthsarana (Sinhala) Indian–Bread Shot (English)</td>
<td>Seed</td>
<td>relieves ear ache, fever, dropsy, dyspepsia.</td>
</tr>
</tbody>
</table>
of *Solanum nigrum* showed the lowest IC\(_{50}\) value and which was lower than that of the standard ascorbic acid.

**Ascorbic Acid Content**

The ascorbic acid content in 100g of plant extracts as measured using the iodometric method revealed that *Amorphophallus campanulatus*, *Solanum nigrum*, *Amaranthus spinosus* and *Canna indica* contains 143.03 ± 1.97mg, 17.16 ± 1.78mg, 8.80 ± 1.81mg, and 0.43 ± 0.01mg respectively. *Amorphophallus campanulatus* showed the highest amount of ascorbic acid in its extract (Figure 2; Table 2).

**DISCUSSION**

In recent years, there has been a worldwide trend towards investigating on natural phytochemicals and quantifying their antioxidant properties. With many synthetic antioxidants exhibiting toxic and or mutagenic effects, the attention has shifted towards the use of naturally occurring antioxidants. Antioxidants have the capability of inhibiting or impairing radical reactions in many neurodegenerative diseases, cancers and AIDS.[16,17] Many plant extracts used in ayurvedha and herbal treatments has reported to have positive curing or controlling effects on the above diseases and many previous investigation have revealed that the many plant extracts does have antioxidant effects and could be therapeutically useful.[18]

During the present study five plants, namely, *Solanum nigrum*, *Amaranthus spinosus*, *Elephantopus scaber*, *Canna indica*, and *Amorphophallus campanulatus* were screened for the antioxidant activity.
their antioxidant properties by evaluating their free radical scavenging activity and also quantifying the ascorbic acid content in the extract.

The model DPPH free radical scavenging assay is an easy method to evaluate antioxidant activity in a relative short time compared to the other methods.[19] DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of the antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses its color stoichiometrically depending on the number of electrons taken up. The color change can be quantified by its decrease of absorbance at wavelength 517nm. The antioxidants exert their DPPH free radical scavenging due to their hydrogen donating ability.[19]

The decreasing order of the IC_{50} Values of the plant extracts were as Canna indica > Amorphophallus campanulatus > Elephantopus scaber > Amaranthus spinosus > Solanum nigrum (Table 2; Figure 1). The results clearly indicated that Solanum nigrum is the plant extract with the best DPPH scavenging activity with an IC_{50}value of 37.63 ± 0.11µg/ml and even better than that of ascorbic acid (40.71 ± 0.13µg/ml). The uses of the plant has been different form country to country and it is reported a wide range of uses as to treat sore eyes, various skin diseases, malaria (Rhodesia), diabetes (Philippines), inflammation of the bladder (Mauritius), headaches, ulcers, wounds (Europe) and many more.[20] (Table 1). The lowest DPPH activity was recorded form Canna indica during this experiment yet the plant extract is widely used for its antioxidant properties in traditional medicinal uses. Previous studies performed on Canna edulis (cold methanol extract) showed an IC_{50} value of 570µg/ml and it is believed that the activity is due to the presence of polyphenols and flavonoids. This plant is also widely used for its antioxidant properties.[21]

Ascorbic acid which is commonly known as Vitamin C is required for the prevention of scurvy and maintenance of healthy skin etc. Its deficiency would also lead to hemorrhage of mucus membrane of the mouth and gastrointestinal tract, anemia, pains in joint.[22] It also plays a major role in activity of the enzyme prolyl hydroxylase which synthesizes 4-hydroxyproline, an amino acid that is required in collagen formation. It is believed that ascorbic acid helps in the enhancement of the immune system. It also scavenges free radicals by acting as a chain breaking antioxidant that impairs the formation of free radical, quenches O_{2}^{-}, and acts as a reducing agent and thereby reducing the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer.[16, 17, 23]

All plant extractsof Amorphophallus campanulatus, Solanum nigrum, Amaranthus spinosus and Canna indica showed significant amount of ascorbic acid in their extracts. However Amorphophallus campanulatus showed the highest amount of ascorbic acid in its extract (Figure 2; Table 2). Previous studies performed in India on the same plant recorded to contain 14.5 µg of ascorbic acid per milligram of extract indicating the values obtained in our experiment to be very much similar.[24] The plant extracts of Amorphophallus campanulatus has reported to be have many medicinal uses as antibacterial, antifungal, and cytotoxic activity. It is also used to relieve pain in acute rheumatism, skin & blood diseases, abdominal pains, tumors, spleen enlargement, asthma and rheumatism.[25] It is also widely studied for its activity against induce oxidative stress[25] and hepatoprotective activity[26] in the recent past and has exhibited promising results for the same.

Correlating the ascorbic acid content with the antioxidant activity as determined by DPPH assay did not show a direct correlation between each other (Figure 2) indicating that ascorbic acid is not, the only substance involved in radical scavenging activity. However extract of Solanum nigrum with its substantially low IC_{50} value for the inhibition of DPPH radicals and Amorphophallus campanulatus for the presence of ascorbic acid has shown promising results as good natural antioxidant. These values greatly supports the investigations results reported on Amorphophallus campanulatus in the resent past for its activity against induce oxidative stress[25] and hepatoprotective activity.[26, 27]

**CONCLUSION**

With the potential of using natural antioxidants as medicines and as food additive the antioxidant research has attracted a prominent place at present. With the reported undesirable effects of synthetic antioxidants and the high cost for such antioxidants, have also encouraged most of the people to use natural antioxidants.

Through this investigation we have shown that all five plants used in this investigation exhibits antioxidant activities with four of them namely to Solanum nigrum, Amaranthus spinosus, Elephantopus scaber, and Amorphophallus campanulatus be having remarkable antioxidant activity with a good potential to be used in therapeutics. Solanum nigrum revealed to be the most potent plant extract with the best radical scavenging activity and a substantial amount of ascorbic acid. These results also elaborate on the ascorbic acid content that could be used effectively
in the traditional or combinational medical practices. Since the results also show that it is not only the ascorbic acid is responsible in the antioxidant activities of these extracts must be further investigated in quantifying the other responsible antioxidant. The results presented in this report will also provide a suitable guide in choosing natural plant by the medical practitioners as natural oxidants treating and controlling diseases.

REFERENCES