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Antioxidative properties of 34 green leafy vegetables

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ABSTRACT

Green leafy vegetables available in Sri Lanka have not been fully exploited, although they are stipulated to be rich sources of natural antioxidant. This study examined the antioxidant properties of thirty four edible green leafy vegetables popular in Sri Lanka. Methanolic extracts of leafy vegetables were analysed for total phenolic, carotene and chlorophyll content, and were evaluated for total antioxidant capacity, reducing power, lipid peroxidation and DPPH radical scavenging assays. The results indicated that these leafy vegetables have remarkable variations in their antioxidant activities. Among the plant materials screened for their antioxidant properties, *Sesbania grandiflora*, *Cassia auriculata*, *Murraya koenigii* Spreng, *Passiflora edulis*, *Gymnema lactiferum* and *Olax zeylanica* showed high carotene content, antioxidant activities and polyphenolics compared to other leaf varieties tested. A good correlation was observed between antioxidant assays and polyphenolics of the leafy vegetables.

1. Introduction

Many chronic diseases such as cancer and cardiovascular diseases represent an increasing proportion of morbidity and mortality in the developing countries, including Sri Lanka. Various research findings have demonstrated that changes in oxygen utilization in the body and increased formation of reactive oxygen species (ROS) contribute to many chronic diseases (Kaliora, Dedoussis, & Schmidt, 2006; Madamanchi, Vendrov, & Runge, 2005). Although an organism is naturally equipped with antioxidant protection systems to cope with the harmful effects of ROS, the endogenous antioxidant defence system is not totally adequate to counteract the oxidative stress (Houston, 2010). Therefore, protection against oxidative stress depends partly on the adequacy of dietary antioxidants (Kaliora et al., 2006). Evidence suggests that phytochemicals from fruits and vegetables, including leafy vegetables, are capable of providing protection against free radicals. Therefore, a greater deal of research has been focused on natural antioxidants and it is necessary to screen the natural sources for their antioxidant potential.

Green leafy vegetables constitute a major part of any balanced diet and contain significant amounts of minerals and

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antioxidant vitamins (Subhasree, Baskar, Keerthana, Susan, & Rajasekaran, 2009). Various research findings have suggested that leafy vegetables have medicinal properties such as anticarcinogenic (Rajesh Kumar et al., 2002), antibacterial (Kubo, Fijita, Kubo, Nehei, & Gura, 2004) and antidiabetic (Kesari, Gupta, & Watal, 2005) effects. These health benefits of green leafy vegetables are attributed, at least in part, to their antioxidants. Recently, research has indicated that green leafy vegetables are rich sources of functional components. The major active antioxidant compounds are polyphenolic constituents and carotenoids, among others (Andarwulan et al., 2012; Deng et al., 2013; Khanam, Oba, Yanase, & Murakami, 2012; Subhasree et al., 2009). Lutein is one of the major carotenoids in green leafy vegetables which shows a marked antioxidant activity (Chandrika, Basnayake, Athukorala, Colombagama, & Goonetilleke, 2010). However, most of the green leafy vegetables available in Sri Lanka represent a class of underexploited plants that are stipulated to be rich sources of natural antioxidants.

A great number of *in vitro* assay methods have been developed to evaluate the efficiency of natural antioxidants. These methods include measurement of phenolic content using Folin–Ciocalteu assay, reducing power assay, total antioxidant capacity assay, lipid peroxidation assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. The above *in vitro* methods can be used for the screening of green leafy vegetables commonly available in Sri Lanka. The purpose of this study was to examine the antioxidant characteristics of thirty four edible green leafy vegetables popular in Sri Lanka. The results of this study can be useful for food industry and in preventive medicine in the development of "natural antioxidants"/nutraceuticals from plant sources.

2. Materials and methods

2.1. Materials

Thirty four types of fresh green leafy vegetable samples were collected from various places in Colombo, Negombo and Makandura areas of Sri Lanka. The plant specimens were taxonomically identified by a botanist (Dr. H.D.D. Bandupriya) and the voucher specimens of the samples were deposited in the herbarium of the Department of Food Science and Technology of Wayamba University of Sri Lanka.

2.2. Reagents

Gallic acid, 2,2-diphenyl-2-picrylhydrazyl, Folin–Ciocalteu reagent, ethanol, methanol, sodium phosphate, ammonium molybdate, potassium ferricyanide, phosphate buffer, trichloroacetic acid, ferric chloride, ferrous sulphate, acetic acid, thiobarbituric acid, sodium dodecyl sulphate, butanol and sodium carbonate were obtained from Sigma Aldrich, St. Louis, MO, USA through Analytical Instrument Pvt Ltd, Colombo, Sri Lanka. All other chemicals used were of analytical grade.

2.3. Preparation of crude extracts

Edible portions of the leaves were cleaned with distilled water and air dried at room temperature (30 ± 2 °C) for 2 hours. The leaves samples were then oven dried at 45 °C to a constant weight. One gram of air dried sample from each of these thirty four leafy vegetables was mixed with 20 mL of methanol and vortexed at high speed for five minutes and then centrifuged (Hettich, EBA 20) for 10 min at 792 g. The extracts were subsequently filtered through a filter paper (Whatman No. 42; Whatman Paper Ltd, Maidstone, UK) and the prepared extracts were stored at –18 °C until assayed within 1 week.

2.4. Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu assay of Singleton, Orthofer, and Lamuela-Raventos (1999) with some modification, as described by Gunathilake and Rupasinghe (2014). About 0.5 mL of extract and 0.1 mL of Folin–Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min in the dark. Then 2.5 mL 7.5% sodium carbonate was added to the mixture and further incubated for 2 hours in the dark at room temperature and then the absorbance was measured at 760 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). The concentration of total phenols was expressed as μg gallic acid equivalents (GAE) per g dry weight of leaf. Gallic acid was used in the construction of standard curve and the linear range used for the calibration curve was 1000–15,000 μg GAE/L.

2.5. Total chlorophyll and carotene contents

The chlorophyll and carotene contents were analysed according to the method described by Türlerinde, Klorofil-A, and Saptanması (1998). The weighed samples were mixed with 96% methanol (50 mL for each gram) for one minute using a vortex. The homogenate was filtered through a filter paper (No: 42 Whatman) and centrifuged using the centrifuge (EBA20) for 10 min at 245 g. The supernatant was separated and the absorbance was read at 470, 653, and 666 nm on UV/VIS spectrometer (SP-3000).

The concentration of each pigment was calculated according to the formulas of Kichtenthaler and Wellburn (1983) and was reported as μg per g dry weight of sample.

Chlorophyll a = 11.75 (A662) – 2.350(A645).
Chlorophyll b = 18.61 (A645) – 3.960 (A662).
Carotene = 1000 (A470) – 2.270 (C_a) – 81.4 (C_b)/227.

where C_a is chlorophyll a and C_b is chlorophyll b.

2.6. DPPH radical scavenging assay

The capacity of prepared extracts to scavenge the 'stable' free radical DPPH was monitored according to the method of Hatano, Kagawa, Yasuhara, and Okuda (1988) with slight modifications. Extracts (100 μL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/VIS spectrometer (SP-3000, OPTIMA INC, Japan) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula.