Industrial Crops and Products 83 (2016) 630–634

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Short communication

Assessment of antioxidant activity, minerals, phenols and flavonoid contents of common plant/tree waste extracts

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ARTICLE INFO

Article history:
Received 7 October 2015
Received in revised form
20 December 2015
Accepted 21 December 2015
Available online 7 January 2016

Keywords:
Agroforestry waste
Essential nutrients
Heavy metals
Polyphenols
DPPH activity

ABSTRACT

Extracts of 25 different plant/tree wastes were screened for their phenol and flavonoid contents, antioxidant activity, nutritional and toxicological elemental composition. The commercial exploitation potential of nutrient and polyphenol-rich tree/plant wastes was also discussed. This study is the first to recommend using non-toxic *Melaleuca diosmifolia* leaf, *Melia azedarach* pod, *Alnus cordata* leaf and *Pinus radiata* cones because they all contain the essential elements (N, P, K, S and Fe) for dietary intake, applications as soil amendments, contaminant biosorbents and substrates for composting or biofertilizer preparation. Fruit peel of *Quercus robur*, *M. diosmifolia* leaf and bark, *Eucalyptus leucoxylon* pod and leaf, *Pyrus ussuriensis* and *Prunus cerasifera* leaf aqueous extracts indicated high phenolic content (35–66 mg GAE/g) and antioxidant activity (70–90%). *A. cordata* and *Morus alba pendula* leaf emerged as a unique source of flavonoids (>95%). There are greater prospects for the green synthesis of metallic nanoparticles using these polyphenol-rich residues.

1. Introduction

One of the most important and overlooked constituents today is 'minerals'. Polyphenolic compounds are ubiquitous in vegetation, constitute an important part of the human diet, and have aroused much interest due to their antioxidant properties. Flavonoids constitute the largest group of plant phenolics and are very effective antioxidants (Maisuthisakul et al., 2007). In recent days, efforts have been made to transform natural wastes into products of commercial utility as they are very rich in bioactive compounds such as vitamins, minerals, amino acids, polyphenols, etc. Among these bioactive compounds, some essential mineral elements play an important role as cofactors in many enzymatic processes involved in humans, plants, animals and soil microbes. Mineral and polyphenol-rich plant materials are of interest to the cosmetic, nutraceutical, remedial and food industries (Kuppusamy et al., 2015). For this reason the search for cost-effective mineral- and phenol-rich natural materials has continued to this day.

To date only limited knowledge is available concerning the use of natural materials especially the abundantly available plant/tree wastes which we dispose of every year at nearly a rate of 24 billion tons (EnviraChar, 2013). Such disposals encompass leaves, fruits, barks, flowers and grass clippings, that are expected to have high levels of minerals and phenolic antioxidants. To date the available literature has not described the full elemental composition and the phenolic ranges of plant/tree wastes. In particular demarcated profiles of the essential, trace and toxic mineral composition of these natural residues are not available. Such information would facilitate their effective utilization. With this in mind, our study was conducted with a primary aim to: firstly, evaluate the elemental composition, phenol, flavonoid contents and antioxidant activity of the so far unexplored, commonly available plant/tree wastes for the first time; and secondly, suggest their potential and practical applications.

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http://dx.doi.org/10.1016/j.indcrop.2015.12.060
0926-6690/

2. Materials and methods

2.1. Chemicals

2,2-diphenyl-1-picryl-hydrazyl (DPPH), Folin-Ciocalteau's reagent, sodium carbonate anhydrous, aluminium chloride, potassium acetate, methanol and nitric acid (HNO_3), all of analytical grade were purchased from Sigma–Aldrich (Steinheim, Germany). They included gallic acid, quercetin and ascorbic acid which serve as standards for spectrophotometric assays of total phenols, flavonoids and antioxidant activity, respectively. All solutions were prepared with Milli-Q water (18 Ω cm^{-1}, Milli-Q, ELGA labwater, UK).

2.2. Plant material

Specimens of 25 different plant/tree wastes consisting of 17 leaves [eucalyptus (*Eucalyptus leucoxylon*), olive (*Olea europaea*), flame tree (*Brachychitona cerifolius*), nerium (*Nerium oleander*), pear (*Syzgium sp.*), cherry plum (*Prunus cerasifera*), coastal tea tree (*Leptospermum laevigatum*), Italian alder (*Alnus cordata*), cockie's tongue (*Templetonia retusa*), casuarina (*Casuarina obesa*), Manchurian pear (*Pyrus ussuriensis*), native hibiscus (*Alyogyne hakeifolia*), bracelet honey myrtle (*Melaleuca armillaris*), desert ash tree (*Fraxinus angustifolia*), green honey myrtle (*M. diosmifolia*), weeping white myrtle (*Morus alba pendula*) and red cottonwood (*Hibiscus tiliaceous rubra*)], 1 bark [bracelet honey myrtle (*M. armillaris*)], 6 fruit/seed pods [eucalyptus (*E. leucoxylon*), oak (*Quercus robur*), cow-itch (*Lagunaria patersonii*), oriental plane (*Platanus orientalis*), white cedar (*Melia azedarach*) and casuarina (*C. obesa*)] and 1 flower [pine (*Pinus radiata*)] that had fallen off from full-grown plant/tree species were collected from Mawson Lakes, South Australia between June and December 2014. Collected residues (1 kg of each specimen) were washed with MQ water to remove the adhering soil particles. Then they were air dried at 40 °C for 72 h, grounded to a fine powder and passed through a 0.5 mm sieve to a uniform powder. The powdered sections were stored in a desiccator with polythene sealing.

2.3. Determination of mineral elements

Analysis of mineral elements was carried out after HNO_3 (70%) acid digestion of the powdered material by Agilent 7500 c (Agilent Technologies, Tokyo, Japan) Inductively-Coupled Plasma Mass Spectrometer (ICP–MS). The C and N contents of the samples were measured using a Trumac (Leco® Corporation, Michigan, USA) carbon–nitrogen–sulphur analyzer (CNS analyzer).

2.4. Extraction procedure for polyphenol compounds

Five mL of boiled water was added to 0.20 g of the sample and the suspensions were left for 24 h in a shaker at room temperature (24 ± 2 °C). The extracts were then centrifuged for 10 min at 3000 rpm and the supernatant collected at 4 °C and used within 24 h to determine phenols, flavonoids and antioxidant activities of the selected plant/tree wastes. All samples were extracted in triplicate.

2.5. Determination of phenolic compounds and antioxidant activity

2.5.1. Total phenols (TP)

TP content was determined by a modified version of the Folin-Ciocalteau's method adapted to micro-scale (Singleton and Rossi, 1965). TP content was evaluated by measuring the variation in absorbance at 660 nm after 45 min of reaction (100 μL of extract + 250 μL of Folin-Ciocalteau phenol reagent + 1 mL of 20% sodium carbonate), by using Synergy™ HT (Bio-Tek® instruments, Inc., Vermont, USA) multi-detection microplate reader. Samples were diluted (1:5) and were quantified using gallic acid (GAE) as standard. Results were expressed as mg of GAE equivalents per g of dry material.

2.5.2. Total flavonoids (TF)

TF content was measured by a modified method described by Khomdram and Singh (2011). The reaction mixture comprised 100 μL of extract, 100 μL of 10% aluminium chloride and 100 μL of 1 M potassium acetate solutions. After 30 min incubation at room temperature, absorbance of the yellow colored mixture was measured at 415 nm using a microplate reader. A standard curve was plotted using different concentrations of quercetin (0–50 μg/mL) and the amount of total flavonoids was calculated as quercetin equivalents in mg per g of dry material.

2.5.3. Screening of the extracts for antioxidant potential—DPPH radical scavenging activity (DPPH inhibition)

DPPH is a widely used stable free radical to evaluate the antioxidant potential of a sample. DPPH radical scavenging activity of the plant/tree waste extracts was evaluated according to a method outlined by Blois (1958) with slight modifications. Briefly, 60 μM of DPPH radical solution in methanol was prepared and 3.9 mL of this solution was mixed with various concentrations of the sample solution. After 30 min, absorbance of the sample (As) was measured at 515 nm. Simultaneously, blank containing 100 μL of water was treated as above and its absorbance was recorded as A_b. The DPPH radical scavenging activity was calculated as follows.

$$\%\text{DPPH inhibition} = \frac{A_b - A_s}{A_b} \times 100$$

2.6. Statistical analysis

All experimental results were reported as mean values ($n = 3$; ± standard deviation). Analysis of variance (ANNOVA) and Tukey's test were carried out using IBM SPSS statistics 20 software package (IBM® Corporation, USA).

3. Results and discussion

3.1. Mineral composition of plant/tree wastes

Monitored metal concentrations are presented in Tables 1 and 2. As can be observed in Table 1, significant variability exists in the elemental composition of different tree wastes tested. These substantial differences are likely to account for their distinct beneficial activities when exploited. The highest levels of C were found in *E. leucoxylon* (506.8 ± 24 mg/g) and *M. diosmifolia* (502.1 ± 49 mg/g) leaves. *M. azedarach* pod and *Hibiscus tiliaceous rubra* leaf recorded substantially higher NPK and Mg followed by leaves of *Morus alba pendula* and *P. cerasifera*. The highest Ca, S and Na concentrations were found in dried leaves of *A. cordata* (52.5 ± 12 mg/g), *A. hakeifolia* (16.3 ± 5.2 mg/g) and *M. armillaris* (12.1 ± 2.6 mg/g), respectively. *P. orientalis* fruit and *M. azedarach* pod exhibited higher concentrations of Cu (Table 2). *M. diosmifolia* leaf showed a very high value of Mg (181 ± 19 μg/g) and Co (0.7 ± 0.8 μg/g) compared to the remaining samples analyzed. Dried leaves of *E. leucoxylon* were a rich source of Ni (5.4 ± 1.5 μg/g). Zn was rich in dried leaves and bark of *M. diosmifolia*. Iron, the most essential mineral in human health and essential for good cardiovascular health, cognitive development and immune function (Trumbo et al., 2001) was the predominant one in *P. radiata* cones (514 ± 24 μg/g) followed by leaves of *M. armillaris* (358 ± 58 μg/g). The amount of a nutritionally essential mineral that helps in preventing diabetes,