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Bioactivities of low-grade green coffee and spent coffee in different *in vitro* model systems

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ABSTRACT

Methanolic extracts of low-grade green coffee (LCB) and spent coffee were analysed for radical-scavenging activity (α,α-diphenyl-β-picrylhydrazyl radical) and oxygen radical absorbance capacity (ORAC). The extracts were also evaluated for anti-tumour (P388 cell assay), anti-inflammatory (J774A.1 cell assay) and anti-allergenic (RBL-2H3 cell line) activities *in vitro*. LCB extract was found to exhibit a radical-scavenging activity of 92.0% followed by spent Arabica (86.9%) and spent Robusta (82.0%) at a concentration of 50 ppm. The antioxidant activity of LCB extract, measured as Trolox equivalents (4416 μM/g) was significantly ($p < 0.05$) higher than that of the spent coffee extracts. However, extracts of spent coffee exhibited significantly ($p < 0.05$) more anti-tumour activity than the LCB extract in terms of cell viability. This could be due to the possible role of brown pigments (melanoidins and phenolic polymers), formed during roasting, which may protect cells from oxidative damage in the biological system. However, both the extracts of LCB and spent coffee showed limited anti-inflammatory and anti-allergic activity. The presence of phenolics and chlorogenic acids in appreciable quantities along with brown pigments makes these coffee by-products a source for natural antioxidants.

1. Introduction

Coffee is one of the world's most popular and widely consumed beverages. Two species are of significant economic importance namely, Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*) (Varnam & Sutherland, 1994). In the recent years, due to the increasing interest in finding physiologically functional foodstuffs, the relation between coffee and health has been extensively investigated (George, Ramalakshmi, & Jagan Mohan Rao, 2008). Coffee is found to exhibit a number of bioactivities, such as antioxidant (Nicoli, Anesec, Manzocco, & Lerici, 1997; Ramalakshmi, Rahath Kubra, & Jagan Mohan Rao, 2008; Rosenberg, 1990), anti-carcinogenic (Giovannucci, 1998; Inoue, Tajima, & Hirose, 1998) and anti-mutagenic activity (Kim & Levin, 1988).

In general, coffee is graded based on the size, colour and percentage of imperfections. Defective coffee obtained after grading is termed as low-grade coffee beans (LCBs), which contain imperfections, such as blacks, dark brown beans, insect-damaged beans, spotted beans, sours, bits and greens (immature beans). LCB are obtained as a result of either improper formation within the fruit or by faulty processing. These beans produce undesirable taste in the beverage, when mixed with graded beans. LCB represents about 15–20% of coffee production on a weight basis and are a problem for disposal.

Specific studies that correlate the presence of defects and quality with respect to physical and chemical characteristics of graded beans in the Brazilian region have been carried out (Franca, Oliveira, Mendonca, & Silva, 2005; Mazzafera, 1999; Ramalakshmi, Rahath Kubra, & Jagan Mohan Rao, 2007). In particular, chlorogenic acid, one of the components in coffee responsible for its antioxidant activity, was found to be high in defective coffee beans, indicating changes in the chemical composition.

Instant coffee is produced from green coffee after roasting, grinding and extraction and concentration of water solubles. After extraction, the remaining residue, co-product, is referred to as "spent coffee". Almost 50% of the world produce is processed for soluble coffee. A major problem encountered by the industry is the disposal of spent coffee. Disposal or utilisation of spent coffee has included sewer discharge, sanitary land fill, incineration, cattle feed, and as fillers and adsorbents in thermosetting material (Boopathy, 1987; Ligo Eugenie, 1970; Navarini et al., 1999; Rizzi & Gutwein, 1994; Sivetz & Desrosier, 1979; Stahl & Turek, 1991).

Since low-grade coffee beans and spent coffee residue are by-products obtained in the coffee industry, an attempt was made to prepare their extracts, for evaluating their bioactivity with

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reference to antioxidant (ORAC assay), anti-tumour (P388 cell assay) anti-inflammatory (J774A.1 cell assay) and anti-allergenic (RBL-2H3 cell degranulation assay) properties, using *in vitro* model systems.

2. Materials and methods

2.1. Chemicals

Reference standards such as caffeine, chlorogenic acid, gallic acid, Folin-Ciocalteu's reagent, α,α-diphenyl-β-picrylhydrazyl radical, butylated hydroxylanisole (BHA), fluorescein sodium salt and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), anti-DNP (dinitrophenyl)-IgE and DNP-HSA (human serum albumin) were purchased from Sigma Chemical Co., St. Louis, MO. Cell proliferation reagent WST-1 was purchased from Takara Bio Inc., Shiga, Japan. 2,2'-Azinobis(2-amidinopropane)dihydrochloride (AAPH), Wortmannin and solvents, such as hexane, methanol and other chemicals, were purchased from Wako Pure Chemical Industries, Kyoto, Japan.

2.2. Plant material

Commercially available low-grade green coffee beans (LCB) were procured from the local market of Mysore, Karnataka, India. LCB (500 g each) were weighed, ground and sieved using a size-18 mesh (650 μm). Sample, in powder form, was packed in low-density polyethylene pouches and preserved at 8–10 °C for further analysis. Two varieties of graded coffee beans, i.e., Arabica plantation (Ar) and Robusta cherry (Rb) were procured from the same local market. Beans were medium roasted and ground to obtain a coarse powder (>650 μm).

2.2.1. Preparation of spent coffee

Two varieties of roasted and ground coffee powder (1 kg) were extracted with de-ionised water in a column, where the temperature of the extraction system was maintained at 92 ± 5 °C. After extracting for 6 h, the spent coffee residue was dried in a hot air oven at 60 °C for 3 h. The product obtained was used for further extraction with suitable organic solvents.

2.3. Moisture and total soluble solids

Moisture content of LCB and spent coffee (Ar and Rb) samples was determined in a hot air oven at 105 ± 2 °C for 48 h (Mazzafera, 1999). Total soluble solids (TSS) content was determined by refluxing the coffee samples (2 g) with hot water (200 ml) for 1 h and then diluting to 500 ml. An aliquot (50 ml) was evaporated to dryness, followed by heating in a hot air oven at 105 ± 2 °C, after which the amount of total soluble solids was calculated (AOAC, 2000).

2.4. Caffeine

LCB and the spent coffee powder were extracted with distilled water along with magnesium oxide for 45 min and filtered through Whatman no. 1 filter paper. The filtrate was extracted with chloroform, the extracts were dried and the absorbance was measured at 275 nm in a spectrophotometer (Cintra 10, GBC, Dandenong, Australia). The quantity of caffeine was calculated using a standard graph prepared from a caffeine reference standard (AOAC, 2000).

2.5. Chlorogenic acids

Chlorogenic acid level of the coffee samples was estimated by a spectrophotometric method. Before and after lead acetate treatment of the coffee extract, absorbance was measured at 325 nm (AOAC 2000) and the chlorogenic acid content was calculated from standard curve.

2.6. Total polyphenols

Total polyphenol content of coffee samples was determined using Folin–Ciocalteu's reagent. The experimental samples (0.5 g) were mixed in 10 ml of methanol: water (70:30, v/v) and heated in a water bath (70 °C) for 10 min. The solution was subjected to centrifugation and the supernatant was separated. Saturated sodium carbonate solution (1.5 ml) and Folin–Ciocalteu's reagent (0.5 ml) were added to the supernatant (0.5 ml). The solution was made up to 10 ml with distilled water. Absorbance of this solution was measured at 765 nm and the total polyphenol content was expressed as gallic acid equivalents (Swain & Hillis, 1959).

2.7. Extraction with solvents

LCB (100 g) and spent coffee powder (100 g) were defatted with hexane (1:6, w/v) for 8 h in a Soxhlet extraction system. The defatted powder was extracted with methanol for 8 h whilst maintaining a material to solvent ratio of 1:8 to 1:12. The extracts were concentrated to dryness using a rotary evaporator at 50 °C under reduced pressure and stored in desiccator for further use.

2.8. Radical scavenging activity

The radical-scavenging activity of the extracts of LCB and spent coffee were evaluated according to the procedure described by Blois (1958) with slight modifications (Jayaprakasha & Jaganmohan Rao, 2000). The extracts and BHA at different concentrations (50, 100 and 200 ppm) were taken in different test tubes. Four millilitres of 0.1 mM methanolic solution of DPPH were added to these tubes and shaken vigorously. The tubes were allowed to stand at 27 °C for 20 min. The control was prepared as above without any extract and methanol was used for the baseline correction. Optical density (OD) of the samples was measured at 517 nm. Radical-scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\%\ \text{Radical scavenging activity} = 100 \times \left(\frac{\text{control OD} - \text{sample OD}}{\text{control OD}}\right)$$

2.9. Oxygen radical absorbance capacity (ORAC) assay

The ORAC value of the extracts was evaluated according to the method of Huang, Ou, Hampsch-Woodill, Judith, and Prior (2002). Extracts (0.2 g) of coffee samples were dissolved in boiling water (8 ml) and maintained at 60 °C for 10 min. The solutions were centrifuged at 3000 rpm for 5 min and the supernatant was used. In brief, 20 μl of sample, which were diluted to appropriate concentration with phosphate-buffered saline (PBS), and Trolox were placed into 96-well plate. For calibration, Trolox (6.25–50 μM) was prepared each day by diluting the stock solution (2 mM). The working solution of fluorescein sodium salt (200 μL, 81.6 nM) was obtained by subsequent dilution of stock solution (8.16 μM) with potassium phosphate buffer (75 mM, pH 7.0). After fluorescein solution was added, fluorescence ($k_{\text{excitation}}$ = 485 nm, k_{emission} = 528 nm) of 0 min ($f_{0\text{min}}$) was measured in a multidetection microplate reader (Powerscan, Dainippon Sumitomo Pharma, Osaka, Japan) equipped with Gen 5 software. AAPH (75 μL) was prepared fresh at a concentration of 200 mM and used for automatic injection. Fluorescence was recorded each minute over 40 min ($f_{2\text{min}} - f_{40\text{min}}$). All samples were analysed in triplicate at three dilutions and the mean value