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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

was taken for ORAC determination. The quantification of the antioxidant activity was based on the calculation of the area under the curve (AUC), as proposed by Cao and Prior (1999). The antioxidant activity by ORAC was calculated as μmol of Trolox equivalents (TE) per gram of sample using the formula:

$$\text{Relative ORAC value} = [(\text{AUC}_{\text{sample}} - \text{AUC}_{\text{blank}})/(\text{AUC}_{\text{Trolox}} - \text{AUC}_{\text{blank}})] \times (\text{TE/g of sample})$$

The experimental data on triplicate analyses are reported.

2.10. Anti-tumour activity of extracts on P388 cell assay

Anti-tumour activity of the extracts was analysed by P388 cell assay, using the method described by Shinmoto, Kimura, Suzuki, and Yamagishi (2001). Extracts (0.1 g) were weighed accurately, mixed with 10 ml of dimethyl sulfoxide (DMSO) and were shaken overnight at room temperature. The solution was centrifuged at 3000 rpm for 5 min, and the supernatant was taken for the assay. Extract was diluted to 2000 ppm concentration with PBS while the concentration of DMSO was kept at 20%.

Briefly, P388 cells were grown in RPMI 1640 (Roswell Park Memorial Institute Medium) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 μg/ml streptomycin, and were kept in an incubator (37 °C, 5% CO_2 and 95% air). Cells were seeded in the culture medium (100 μl) at a concentration of 5×10^4 cells/well in a microtitre plate (tissue culture grade, 96-wells, flat bottomed, BD Falcon™; BD Biosciences, San Jose, CA). Coffee extracts were added into the wells, separately along with DMSO, which acts as a negative control for the cell growth inhibition. After 48 h of incubation, 10 μl of WST-1 reagent was added per well, and the cells were incubated for 4 h at 37 °C. The absorbance was measured at 490–650 nm in an ELISA reader (Molecular Devices $\text{Thermo}_{\text{max}}$ Microplate Reader, MDS Inc., Toronto, Canada).

2.11. Anti-inflammatory activity studies using J774A.1 cell assay

Anti-inflammatory activity of the extracts was determined through tumour necrosis factor (TNF-α) which is an important cell-mediated immune response activity. J774A.1 mouse macrophage cell line [NIHS (JCRB), Tokyo, Japan; Cell No: JCRB 9108] was selected for the study, and was maintained in 10% FCS with Dulbecco's modified Eagle's medium (DMEM; Sigma, MD, USA)) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (37 °C, 5% CO_2 and 95% air). The methodology described by Herath, Ishikawa, and Yamaki (2003) was followed.

In brief, sample (0.1 g) solutions were prepared separately from all the extracts, followed by dissolution in 10 ml of DMSO. These extracts were further diluted with Hank's solution to get final concentrations of 1, 3 and 10 μg/ml in the assay medium. Under these conditions, none of the solvents altered the production of TNF-α without the stimulation of lipopolysaccharide (LPS), procured from *Escherichia coli* 0111:B4 (Difco, Detroit, MI). The cell suspension (200 μl) at a concentration of 2.5×10^5 cells/ml was placed in 96-well cultured plate and was incubated for 18 h. After incubation, culture medium was removed, and test samples (1, 3 and 10 μg/ml) and LPS (final concentration, 1.0 μg/ml) were added to each well and incubated for a further 3 h. Supernatant was then collected and assayed for TNF-α content using mouse TNF-α ELISA kit (eBioscience, San Diego, CA, USA). The inhibition of plant extracts against TNF-α production was calculated from the production of vehicle.

2.12. Anti-allergic activity on RBL-2H3 cell line

Rat basophilic leukemia RBL-2H3 [NIHS (JCRB), Tokyo, Japan; Cell No.: JCRB 0023] cells are mucosal mast cell type, which is a major model for the study of anti-allergenic activity of foodstuffs (Watanabe, Shinmoto, & Tsushida, 2005; Yamashita et al., 2000).

RBL-2H3 cells were grown in Dulbecco's modified Eagles Medium (DMEM) containing 10% foetal calf serum (FCS). The cells were inoculated into a 24 well plate (1 ml of 2.5×10^5 cells) and were cultured overnight at 37 °C in an atmosphere of 5% CO_2 and 95% air. Mouse monoclonal anti-DNP IgE solution (500 μl, 50 ng/ml solved in modified Tyrode buffer (MT)) was added to each well. After 2 hours of incubation, antibody solution was removed and washed two times with modified Tyrode (MT) buffer. Extracts were diluted to different concentrations (100, 1000, **1000** ppm) with MT buffer and were added to each experimental well (490 μl). Wortmannin solution (5 μM) was used as the positive comparison. After 10 minutes of incubation, DNP-labelled human Serum albumin (final concentration 50 ng/ml) was added to all the wells and incubated for 30 min. The supernatant was collected, and the cells were lysed with 500 μl of 0.1% (w/w) Triton X-100. The supernatant and the cell lysate (50 μl) were transferred to 96-well ELISA plates and were mixed with 100 μl of 0.1 M citrate buffer (pH 4.5) containing 3.3 mM *p*-nitrophenyl-2-acetamide-β-D-glucopyranoside. The mixture was incubated for 25 min at 37 °C. The reaction was stopped by adding 100 μl of 2 M glycine buffer (pH 10.0), the absorbance was measured in a microplate reader (Biorad Model 550, Bio-Rad Laboratories, Hercules, CA), and the degranulation was calculated according to Demo et al. (1999).

2.13. Statistical analysis

All the analyses were carried out in triplicate, and the results are shown as mean value and standard deviation. The data were analysed by analysis of variance and were compared by Tukey's difference test; the 5% level being used to determine the significance.

3. Results and discussion

3.1. Chemical components

Total soluble solids (TSS) of roasted coffee powder obtained from Arabica plantation and Robusta cherry were found to be 23.8 and 31.9% and 31.0% in LCB (Table 1). The present results are in agreement with the values reported in the literature (Ramalakshmi, Prabhakhakara Rao, Nagalakshmi, & Raghavan, 2000; Sivetz & Desrosier, 1979). There was no significant difference between the varieties for TSS. There was an extraction efficiency of 56.8% in Arabica plantation, while it was 77.4% in the case of Robusta cherry.

As expected, the extracts of LCB possessed higher phenolics and chlorogenic acids (21.9 and 34.2%) than the spent coffee extracts of both Arabica and Robusta. The reasons could be (i) the degradation of these compounds during roasting and (ii) release of these compounds into the water extract during the preparation of instant coffee (Table 2).

3.2. Radical-scavenging activity

The free radical-scavenging activity of the extracts was tested using DPPH model system and the results are presented in Table 3. The principle involved in this method is that the antioxidants react with the stable free radical, i.e., α,α-diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α,α-diphenyl-β-picrylhydrazine with discoloration. The degree of discoloration indicates the scavenging potential of the antioxidant sample/extracts (Abdille, Singh, Jayaprakasha, & Jena, 2005). It is observed that LCB extract was found to exhibit maximum radical-scavenging activity (92.0%), followed by spent Arabica (86.9%) and spent Robusta

Table 1
Chemical composition (%) of coffee samples.

Sample	Total soluble solids	Chlorogenic acids	Caffeine	Total polyphenols*
Low-grade green coffee (LCB)	31.0 ± 0.3[a]	8.5 ± 0.0[a]	1.7 ± 0.0[b]	4.5 ± 0.0[a]
R&G Arabica plantation (Ar)	23.8 ± 0.5[b]	2.7 ± 0.0[c]	1.6 ± 0.0[b]	3.5 ± 0.1[b]
Spent coffee (Ar)	10.3 ± 0.2[c]	1.4 ± 0.0[d]	0.5 ± 0.0[c]	1.3 ± 0.0[c]
R&G Robusta cherry (Rb)	31.9 ± 0.89[a]	4.0 ± 0.1[b]	2.4 ± 0.0[a]	4.1 ± 0.1[ab]
Spent coffee (Rb)	7.2 ± 0.3[c]	1.2 ± 0.0[d]	0.2 ± 0.0[c]	1.0 ± 0.0[c]

* Gallic acid equivalents; values are mean ± SD of triplicate analysis. Values not having similar superscripts in the same column are significantly ($p < 0.05$) different.

Table 2
Major antioxidant components (%) of the extracts of LCB and spent coffee.

Sample	Caffeine	Chlorogenic acids	Total phenolics*
LCB	8.3 ± 0.4[a]	34.2 ± 0.3[a]	21.9 ± 0.5[a]
Spent coffee (Ar)	2.5 ± 0.2[b]	5.62 ± 0.0[b]	6.32 ± 0.0[b]
Spent coffee (Rb)	1.0 ± 0.3[c]	4.87 ± 0.0[c]	4.81 ± 0.0[c]

* Gallic acid equivalents; Values are mean ± SD of triplicate analysis. Values not having similar superscripts in the same column are significantly ($p < 0.05$) different.

Table 3
Radical-scavenging activity (%) of extracts of LCB and spent coffee at different concentrations (ppm).

Sample	200	100	50
Low-grade coffee	95.2 ± 4.5	94.3 ± 4.9[ab]	92.0 ± 4.7
Spent coffee (Ar)	89.2 ± 3.9	87.9 ± 4.1[ab]	86.9 ± 5.0
Spent coffee (Rb)	84.3 ± 5.9	83.8 ± 5.1[b]	82.0 ± 5.0
BHA	95.5 ± 4.4	95.1 ± 3.1[a]	95.1 ± 4.0

Values are mean ± SD of triplicate analysis.
Values not having similar superscripts in the same column are significantly ($p < 0.05$) different.

Table 4
Trolox values of extracts of LCB and spent coffee.

Sample	Trolox equivalents (μmol Trolox/g)
Low-grade coffee	4416 ± 215[a]
Spent coffee (Ar)	1821 ± 345[c]
Spent coffee (Rb)	2594 ± 71[b]

Values are mean ± SD of triplicate analysis.
Values not having similar superscripts in the same column are significantly ($p < 0.05$) different.

(82.0%) at a concentration of 50 ppm. However, there was no significant difference in radical-scavenging activity between the extracts, or between different concentrations (50, 100 and 200 ppm) of each extract. Scavenging activity of the water extract obtained from roasted coffee residues was reported as 95.4% at a concentration of 0.2 mg/ml by Yen, Wang, Chang, and Duh (2005). They described that apart from polyphenolic compounds, Maillard reaction products formed during roasting are also responsible for the scavenging activity of coffee extracts. Therefore, the extracts obtained from the spent coffee residues of both varieties showed relatively higher scavenging activity, even though the quantity of chlorogenic acid is considerably lower (Table 2).

3.3. ORAC assay

Accurate measurement of antioxidant capacity requires the measurement of both inhibition degree and inhibition time, since the reaction among free radical, substrate and antioxidant is very complicated, which makes it impossible to use a fixed equation to express the kinetic order. Oxygen radical absorbance capacity (ORAC) is the only method so far that combines both inhibition time and degree of inhibition into a single quantity. The ORAC assay measures the ability of antioxidant compounds to inhibit the loss of fluorescein induced by the peroxy radical generated from AAPH.

Table 4 shows the values obtained in terms of Trolox equivalent (TE) by the ORAC assay method. Green coffee extract showed higher activity than spent coffee extracts. It is reported (Wen, Takenaka, Murata, & Homma, 2004) that the green coffee extract obtained from *Coffea robusta* showed a value of 40 μmol Trolox eq/100 g, whereas the green coffee extract obtained using LCB in this study showed higher activity (4416 μmol Trolox eq/g). In the present study Soxhlet extraction was followed using methanol as an extraction solvent after defatting with hexane, whereas Wen et al. (2004) refluxed the green coffee powder with hot water and the resultant extract was lyophilised before evaluating its antioxidant properties. Leslie (2007) reported that the whole coffee berry extract exhibited an antioxidant capacity 10 times more than that of green tea by ORAC assay method and further suggested that the antioxidant activity is due to the presence of polyphenols, especially chlorogenic acid, proanthocyanidins, quinic and ferulic acids.

There is a significant ($p < 0.05$) difference in Trolox value between the extracts of spent Robusta and spent Arabica. Extract of spent Robusta showed more Trolox activity than spent Arabica extract. An earlier report Castillo et al, 2005 reveals that the brews obtained from *Coffea robusta* showed a higher ORAC value than brews from *Coffea arabica*.

3.4. Anti-tumour activity

Several epidemiological studies have investigated whether coffee consumption induce or promote cancer, although the question remains unclear. Many studies have revealed the protective association between coffee consumption and the risk of certain cancers (Nishi, Ohba, Hirata, & Miyake, 1996; Schilter, Cavin, Tritscher, & Constable, 2001). In our investigation it was found that there was an inhibition of P388 cell growth by all the coffee extracts. Cell viability was reduced to 50.1 ± 3.6% by LCB extract whereas the extracts obtained from spent Arabica plantation and Robusta cherry exhibited significantly ($p < 0.05$) lower values of 20.6 ± 5.3% and 19.2 ± 4.4%, respectively (Fig. 1). There was not much difference between both the varieties of spent coffee extracts on inhibition of cell viability. However, both the spent coffee extracts showed more anti-tumour activity than green coffee extracts, although the amount of polyphenols and chlorogenic acids is greater in green coffee extract. This may be due to the possible role of brown pigments (melanoidins and phenolic polymers), formed during the roasting process, which protect cells from oxidative damage (Wen et al., 2004). Many animal studies also support the anti-carcinogenic property of coffee, which is mainly due to the presence of two diterpenes namely cafestol and kahweol (Cavin et al., 2002).

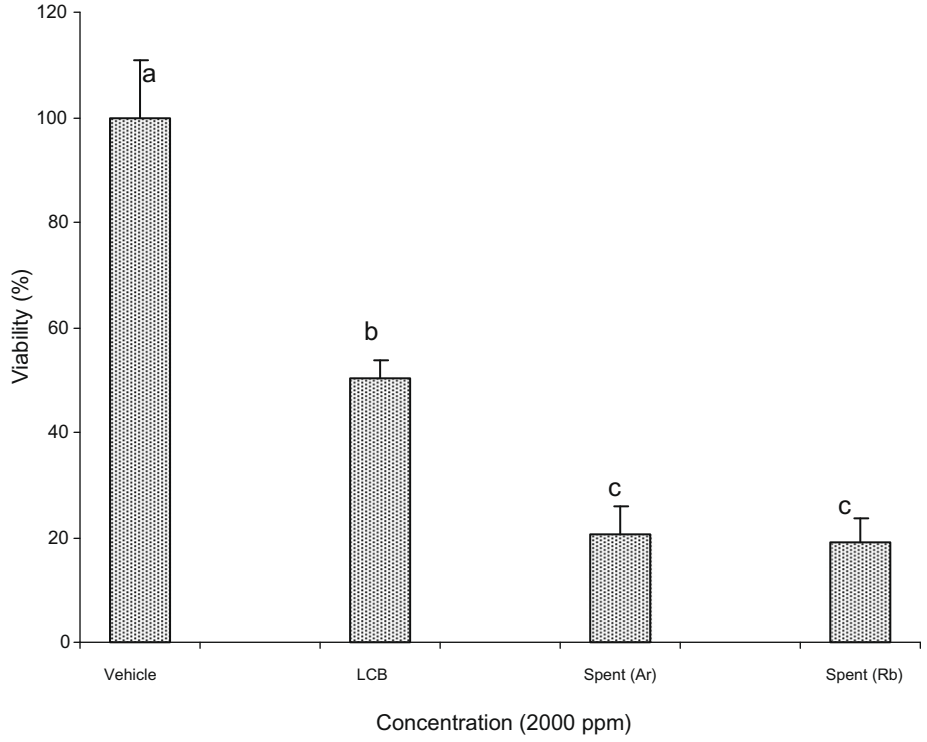

Fig. 1. Anti-tumour activity of coffee extracts by P388 assay. Values are mean ± SD of triplicate analysis, Values not having similar superscripts are significantly ($p < 0.05$) different.

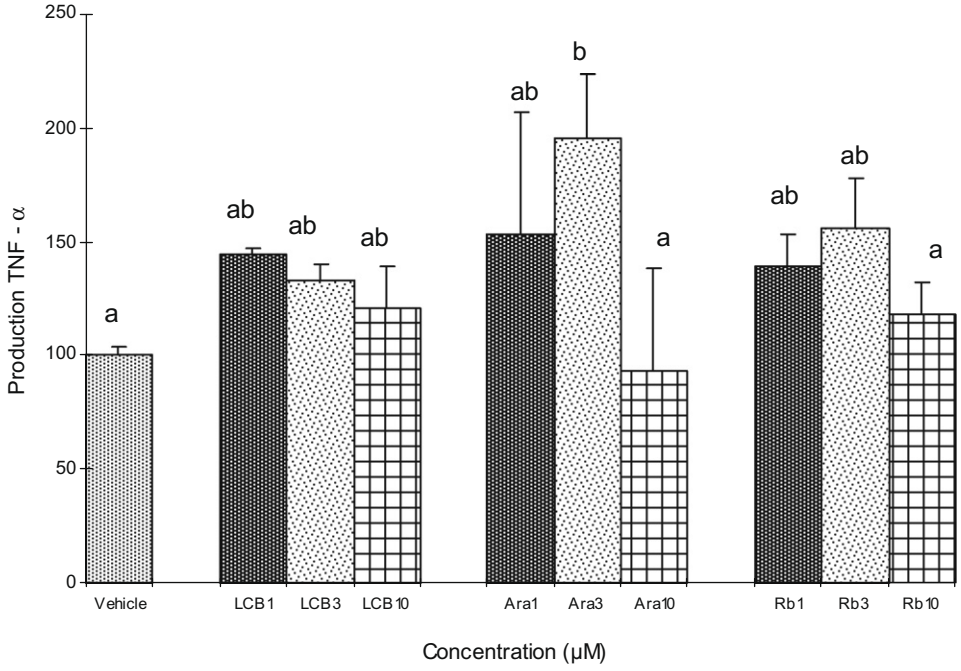

Fig. 2. Anti-inflammatory activity of coffee extracts by J774A1 cell assay. Values are mean ± SD of triplicate analysis, Values not having similar superscripts are significantly ($p < 0.05$) different.

3.5. Anti-inflammatory activity

Researchers are attempting to screen agri-horticultural produce that contains polyphenols, which shows potent anti-inflammatory activity. Inflammation involves a complex web of intra- and intercellular cytokine signals. TNF-α, an inflammatory mediator is one of the most important pro-inflammatory cytokines produced by activated monocytes and macrophages. TNF-α is secreted during the early phase of acute chronic diseases such as asthma, rheumatoid arthritis, septic shock and allergic diseases.

There are not many reports on the anti-inflammatory activity of coffee. However there are reports relating polyphenols and anti-

inflammatory response (D'Alessandro et al., 2003). Though all the coffee extracts have a substantial quantity of polyphenols, none of the extracts showed anti-inflammatory activity against the LPS-induced production of TNF-α (Fig. 2).

3.6. Anti-allergic activity

Food allergy is generally classified as an immediate hypertensive (Type I) allergy. Mast cells or basophils play a central role in immediate allergic reactions mediated by immunoglobulin (Ig) E. Binding multivalent allergens to specific IgE receptors on the surface of mast cells or basophils lead to the release of inflammatory mediators, such as histamine, arachidonic acid metabolites and cytokines. In this study, rat basophilic leukemia (RBL-2H3) cell line was selected to estimate the degranulation inhibitors. Earlier reports say that chlorogenic acids are the important allergic constituents of green coffee bean (Freedman, Shulman, & Krupey 1964). A recent study (Yamashita et al., 2000) reported that polyphenolic compounds from tea inhibit histamine release from mast cells, thereby showing anti-allergenic activity. Extracts obtained from spent coffee of both the varieties could inhibit histamine release (Fig. 3), and an increase in the concentration of the spent extracts increased the inhibitory effect of histamine release significantly ($p < 0.05$). There was not much difference in the anti-allergenic activity between the varieties of spent coffee. At a concentration of 10000 ppm, extracts of both the varieties of spent coffee could inhibit the release of histamine to less than 10% compared to the negative control, which could inhibit around 56%. However, extract of LCB could not inhibit histamine release even at 10000 ppm. When the pure compounds, namely chlorogenic acid and caffeine, were tested, chlorogenic acid inhibited histamine release better than caffeine (Fig. 4). At 10,000 ppm, the effect on the inhibition of histamine release is significantly more for the extracts of spent coffee of both the varieties than the extract of LCB, although the

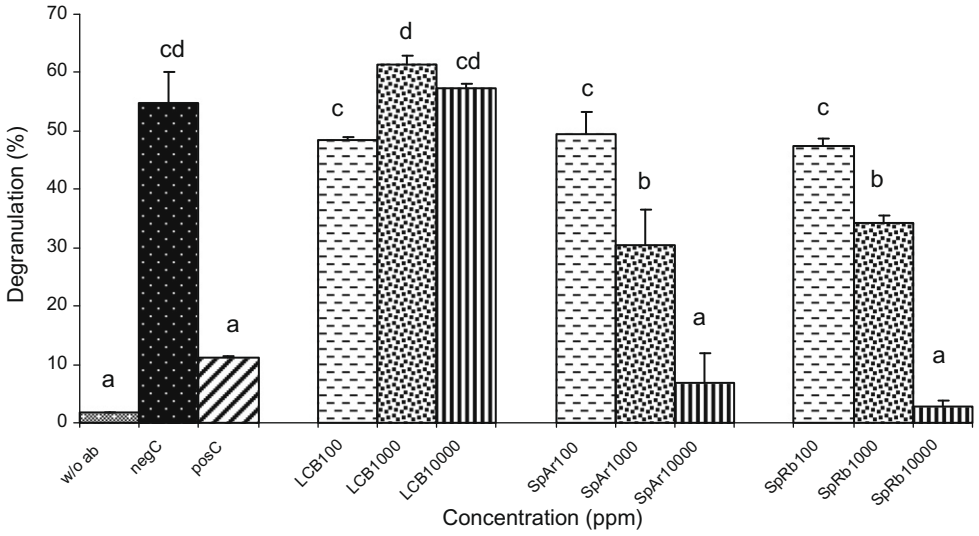

Fig. 3. Anti-allergenic activity of coffee extracts Values are mean ± SD of triplicate analysis, Values not having similar superscripts are significantly ($p < 0.05$) different.

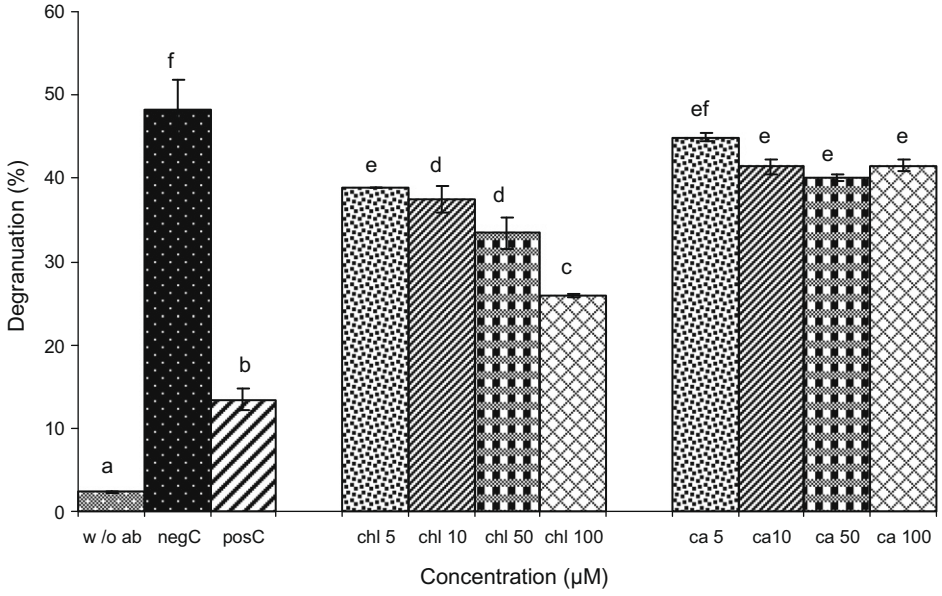

Fig. 4. Anti-allergenic activity of chlorogenic acid and caffeine. Values are mean ± SD of triplicate analysis, Values not having similar superscripts are significantly ($p < 0.05$) different.

amount of chlorogenic acid in spent coffee extracts is significantly ($p < 0.05$) less than in the LCB extract. This result shows that the anti-allergenicity in terms of inhibition of histamine release is due to chlorogenic acid content, which is the major polyphenol present in coffee. The present result is in good agreement with an earlier report (Layton, Panzani, & Corse,1966), where it was reported that coffee-specific allergenicity is not entirely due to the chlorogenic acids but due to the protein present as a contaminant in the coffee extracts.

4. Conclusion

The present study focused on the evaluation of bioactivity of the extracts obtained from LCB and spent coffee. Methanol extract of LCB contains total phenolics (21.9%), chlorogenic acid (34.2%) and caffeine (8.3%). Although there was not much difference in the radical-scavenging activity between the extracts of LCB and both the varieties of spent coffee, there is an increase in the Trolox value for LCB extracts. The extracts obtained from spent coffee exhibited more anti-tumour activity than the extract of LCB when analysed in P388 cell line. However, both the extracts of LCB and spent coffee did not show much activity for anti-inflammatory properties. Extracts of spent coffee of both the varieties possessed anti-allergic activity in terms of the inhibition of histamine release. This study provides evidence for radical-scavenging (RSA), oxygen radical absorbance capacity (ORAC), anti-tumour and anti-allergenic activities of the extracts of LCB and spent coffee. The presence of phenolics, chlorogenic acids and brown pigments, which are the compounds responsible for these activities, in appreciable quantity, makes these by-products a source of natural antioxidants. Incorporation of these active molecules into food systems may require further safety studies such as toxicological effect, dosage level and carry-through effect.

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References

Abdille, Md. H., Singh, R. P., Jayaprakasha, G. K., & Jena, B. S. (2005). Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chemistry, 90*, 891–896.

AOAC. (2000). *Official methods of analysis of AOAC international*, 17th ed. Gaithersburg, MD, USA, Caffeine – 965.25; Chlorogenic acid – 957.04; Soluble solids – 973.21.

Blois, M. S. (1958). Antioxidants determination by the use of a stable free radical. *Nature, 4617*, 1199–1200.

Boopathy, R. (1987). Effect of temperature on anaerobic fermentation of coffee waste. *Asian Environment, 9*, 13–18.

Cao, G., & Prior, R. L. (1999). Measurement of oxygen radical absorbance capacity in biological samples. *Methods in Enzymology, 299*, 50–62.

Castillo, M. D., Gordon, M. H., & Ames, J. M. (2005). Peroxyl radical-scavenging activity of coffee brews. *European Food Research and Technology, 221*, 471–477.

Cavin, C., Holzhaeuser, D., Scharf, G., Constable, A., Huber, W. W., & Schilter, B. (2002). Cafestol and kaweol, two coffee specific diterpenes with anticarcinogenic activity. *Food and Chemical Toxicology, 40*, 1155–1163.

D'Alessandro, Tracy, Prasain, J., Benton, M. R., Bottling, N., Moore, R., Darley-Usmar, V., et al. (2003). Polyphenols, inflammatory response and cancer prevention: Chlorination of isoflavones by human neutrophils. *The Journal of Nutrition, 133*, 3773S–3774S.

Demo, S. D., Masuda, E., Rossi, A. B., Throndset, B. T., Gerard, A. L., Chan, E. H., et al. (1999). Quantitative measurement of mast cell degranulation using a novel flow cytometric annexin-V binding assay. *Cytometry, 36*, 340–348.

Franca, A. S., Oliveira, L. S., Mendonca, J. C. F., & Silva, X. A. (2005). Physical and chemical attributes of defective crude and roasted coffee beans. *Food Chemistry, 90*, 89–94.

Freedman, S. O., Shulman, R., & Krupey, K. (1964). Antigenic properties of chlorogenic acid. *The Journal of Allergy, 35*, 97–107.

George, S. G., Ramalakshmi, K., & Jagan Mohan Rao, L. (2008). A perception on health benefits of coffee. *Critical Reviews in Food Science and Nutrition*, 48, 464-486.

Giovannucci, E. (1998). Meta-analysis of coffee-consumption and risk of colorectal cancer. *American Journal of Epidemiology, 147*, 1043–1052.

Herath, H. M. T., Ishikawa, Y. T., & Yamaki, K. (2003). Inhibitory effect of some flavonoids on Tumor Necrosis Factor-α production in Lipopolysaccharide-stimulated mouse macrophage cell line J774.1. *Journal of Medical Food, 6*, 365–370.

Huang, D., Ou, B., Hampsch-Woodill, M. F., Judith, A., & Prior, R. L. (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agricultural and Food Chemistry, 50*, 4437–4444.

Inoue, M., Tajima, K., & Hirose, K. (1998). Tea and coffee consumption and the risk of digestive tract cancers: Data from a comparative case-referent study in Japan. *Cancer Causes and Control, 9*, 209–216.

Jayaprakasha, G. K., & Jaganmohan Rao, L. (2000). Phenolic constituents from Lichen Parmotrema stuppeum (Nyl.) Hale and their antioxidant activity. *Zeitschrift fur Naturforschung C-A Journal of Biosciences, 55C*, 1018–1022.

Kim, J., & Levin, R. E. (1988). Mechanism of caffeine repression of mitomycin C induced reversion in Salmonella typhimurium strain TA94. *Microbiols, 53*, 181–190.

Layton, L. L., Panzani, R., & Corse, J. W. (1966). Nondiffusible allergenic contaminant isolated from samples of chlorogenic acid causing allergic reactions: Pure chlorogenic acid not an allergen. *The Journal of Allergy, 38*, 268–275.

Leslie, B. (2007). Coffee berry extract: The next big thing. *Skin and Aging, 15*, 80–82.

Ligo Eugenie. (1970). *Waste coffee grounds as fillers in thermosetting materials.* US 3, 499, 851, 10 Mar 1970.

Mazzafera, P. (1999). Chemical composition of defective coffee beans. *Food Chemistry, 64*, 547–554.

Navarini, L., Gilli, R., Gombac, V., Abatangelo, A., Bosco, M., & Toffanin, R. (1999). Polysaccharides from hot water extracts of roasted arabica beans: Isolation and characterization. *Carbohydrate Research, 40*, 71–81.

Nicoli, M. C., Anesec, M., Manzocco, L., & Lerici, C. R. (1997). Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensmittel-Wissenschaft und Technologie, 30*, 292–297.

Nishi, M., Ohba, S., Hirata, K., & Miyake, H. (1996). Dose–response relationship between coffee and the risk of pancreas cancer. *Japanese Journal of Oncology, 26*, 42–48.

Ramalakshmi, K., Prabhakhakara Rao, P. G., Nagalakshmi, S., & Raghavan, B. (2000). Physico-chemical characteristics of decaffeinated coffee beans obtained using water and ethyl acetate. *Journal of Food Science and Technology, 37*, 282–285.

Ramalakshmi, K., Rahath Kubra & Jagan Mohan Rao, L. (2007). Physico-chemical characteristics of green coffee: Comparison of graded and defective coffee beans. *Journal of Food Science, 72*, S-333–S-337.

Ramalakshmi, K., Rahath Kubra & Jagan Mohan Rao, L. (2008). Antioxidant potential of low grade coffee beans. *Food Research International, 41*, 96–103.

Rizzi, G. P., & Gutwein, R. W. (1994). *Roast and ground coffee with defatted spent coffee grounds.* US 5, 328, 708, 12 July 1994.

Rosenberg, L. (1990). Coffee and tea consumption in relation to the risk of large bowl cancer: A review of epidemiological studies. *Cancer Letters, 52*, 163–171.

Schilter, B., Cavin, C., Tritscher, A., & Constable, A. (2001). Coffee and safety considerations. In R. J. Clarke & O. G. Vitzthum (Eds.), *Coffee: Recent developments* (pp. 165–183). London: Blackwell Science.

Shinmoto, H., Kimura, T., Suzuki, M., & Yamagishi, K. (2001). Anti-tumor activity of vegetables harvested in Thoku region. *Nippon Shokuhin Kagaku Kaishi, 48*, 787–790.

Sivetz, M., & Desrosier, N. (1979). *Coffee technology*. Connecticut: AVI Publication Co. Inc., Westport. pp. 518–524.

Stahl, H., & Turek, E. (1991). Acid hydrolysis of spent coffee grounds to produce D-mannose and D-mannitol. In *Proceedings of the 14th ASIC Colloquium (Paris) ASIC Paris* (pp. 339–348), France.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica.* I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*, 63–68.

Varnam, A. H., & Sutherland, J. P. (1994). *Beverages: Technology, chemistry and microbiology*. Boundary Row, London: Chapman and Hall. pp. 191–255.

Watanabe, J., Shinmoto, H., & Tsushida, T. (2005). Coumarin derivatives from estrogen and thyme as inhibitors of chemical mediator release from RBL-2H3 cells. *Bioscience Biotechnology and Biochemistry, 69*, 1–6.

Wen, Xu., Takenaka, M., Murata, M., & Homma, S. (2004). Antioxidative activity of a zinc-chelating substance in coffee. *Bioscience Biotechnology and Biochemistry, 68*, 2313–2318.

Yamashita, K., Suzuki, Y., Matsui, T., Yoshimaru, T., Yamaki, M., Suzuki-Karasaki, M., et al. (2000). Epigallocatechin gallate inhibits histamine release from rat basophilic leukemia (RBL-1H3) cells: Role of tyrosine phosphorylation pathway. *Biochemical and Biophysical Research communications, 274*, 603–608.

Yen, W. J., Wang, B. S., Chang, L. W., & Duh, P. D. (2005). Antioxidant properties of roasted coffee residue. *Journal of Agricultural and Food Chemistry, 53*, 2658–2663.