Pharmacognosy Research [Phcog Res]
Vol 1, Issue 5, Sep-Oct, 2009 Page 245-249
(An Official Publication of Pharmacognosy Network Worldwide)
Received: Jul 10, 2009
Modified: Jul 22, 2009
Accepted: Aug 29, 2009

PHCOG RES.: Research Article

Comparison Between the Antioxidant Properties of Slurry of the Coffee (*Coffea Arabica*) and Coffee Beverage

Rocha, **Bruno Alves***; **Batista**, **Bruno Lemos**; **Duarte**, **Stella Maris da Silveira**; **Dos Santos**, **Marcelo Henrique**.

Departamento de Farmácia, Laboratório de Fitoquímica e Química Medicinal, Universidade Federal de Alfenas - UNIFAL-MG, Rua Gabriel Monteiro da Silva 714, Alfenas - MG, Brasil, acidpharma@yahoo.com.br

** Bruno Alves Rocha : acidpharma@yahoo.com.br*

ABSTRACT

Residues of coffee called slurry are totally discarded after preparing coffee beverages and they do not present any potential use. These represent about 15–20% of coffee production on weight basis and attention are giving in how explored them. Coffee residues and beverage were evaluated, after extraction with different solvents such as hexane, ethyl acetate, dichloromethane, ethanol and methanol. Antioxidant potential from extracts were evaluated then, for this purpose, was used *in vitro* models such as radical scavenging activity (1,1-diphenyl-2-picrylhydrazyl radical, DPPH·), reducing power activity and chelating capacity. The highest yields were obtained for dichloromethane (B), ethyl acetate (C) and methanol (I). B extract exhibits the maximum radical scavenging activity (91.8%) followed by C extract (85.6%) and then, I extract (57.4%) each one at 200 mg L^{-1}. For reducing power activity the extracts B, C, I, ethanol (H) and ethyl acetate (E) showed 85.4%, 71.6%, 36.2%, 19.3% and 12.4% of antioxidant activity, respectively, at 1000 mg L^{-1}. The chelating capacity for I, ethanol (F), C and B extracts was 14.8%, 14.7%, 9.9% and 4.3% when compared to etilendiamintetracetic acid (EDTA) chelating capacity. The high antioxidant potentials observed for the extracts of the slurry is due to the presence of phenolic compounds including chlorogenic acids which make them more suitable as a source of natural antioxidant.

Keywords: Antioxidant Activity, *Coffea Arabica* Roasted coffee, Slurry of coffee.

INTRODUCTION

Coffee is one of the most widely consumed beverages in the world, because of its pleasant aroma and taste, its pharmacological effects and, above all, its stimulatory effects on mental and physical activity (1).

In Brazil its preparation consist in putting the coffee powder in a filter paper and shed hot water through it (between 90°C and 100°C), recovering the drink in a container. The coffee powder after this extraction is called slurry or residue of coffee ground (2). In Brazil, coffee cherries are generally processed using the dry method, resulting in coffee husk, which is rich in organic matter and nutrients. It also contains compounds such as caffeine, tannins and polyphenols. The composition of coffee husk is different from coffee pulp (3).

Coffee beans, including roasted coffee beans are rich in phenolics, particularly chlorogenic acids and other hydrocinnamate-quinic acid derivatives. The slurry coffee might be upgrade by extracting the phenols, which it could be used as food preservatives or dietary supplements for disease prevetion. Dietary phenolics including plant food derived such as flavonoids and phenolic acids, have been suggest as a physiological antioxidant and play a role in the lowering the incidences of coronary heart disease, thrombotic and atherogenic process in humans (4).

Slurry of coffee is the residue of the coffee beverage processing. With improvements of coffee beverage

processing, the volume of coffee residue produced dropped from 1.86 kg to 0.91 kg for each kilogram of coffee beverage made (5). The volume of coffee residues is decreased, however, their disposal still become a matter of great concern. Hence, more rational uses of roasted coffee residues have become necessary. Many attempts have been made to use roasted coffee residues as a fertilizer, animal feed, or substitutes for industrial materials however the best confirmed use of coffee residues is as a fuel (5-6).

The beverage is a very complex mixture of several hundred chemicals which are either naturally occurring or else induced by the roasting process. To examine coffee, caffeine has usually attracted most attention. Except caffeine, the bioactivity and the pharmacological effects as a result of naturally occurring phenolic compounds and Maillard reaction products (MRPs) developed during the roasting process (6) are still unclear. Experimental evidence has shown that coffee has high antioxidant activities (7). Several authors (6, 8–12) have attributed the strong antioxidant properties of coffee beverage to the presence of both naturally occurring phenolics such as chlorogenic acids, caffeic, ferulic and cumaric acid and heat-induced polyphenoltype structures which are formed due to nonenzymatic browning reactions during roasting. Also, caffeine seems to contribute to the overall antioxidant activity of coffee. In fact, Devasagayam et al. (13) showed caffeine as a protector agent of membranes of oxidative damage. In contrast, some literature has reported that roasted coffee has been found to possess mutagenic activity (7) and prooxidant activity (14). However, the same research group also noted that roasted coffee could act as a potent antioxidant and inhibit lipid peroxidation in a model system (14).

Therefore, the aim of this present work was to evaluate the antioxidant activity of roasted coffee residues in light roasting degree and to compare with coffee beverage, evaluating a possible destine for rational uses of residue slurry of coffee.

MATERIALS AND METHODS

Reagents and equipments

All solutions were prepared with reagents of analytical grade and purified water. Hexane, methanol, ethanol, ethyl acetate, dichloromethane, phosphate, potassic ferricyanide, ferric chloride and trichloroacetic acid were obtained from Merck Chemical Industries (Rio de Janeiro, RJ, Brazil). For chelating capacity ferrozine (3-(2-pyridyl)-5,6-bis (4-phenyl acid-sulfonic) 1,2,4-triazine) and other reagents used were purchased from Bioclin (Belo Horizonte, MG, Brazil). 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), ascorbic acid, dimethyl-sulfoxide and etilendiamintetracetic acid (EDTA) were obtained from Sigma Chemical (St. Louis, MO, USA).

Measurements were made using a Shimadzu UV-vis 1610 PC spectrophotometer. It was used also a Thermomix modelo 18U double boiler (Germany) for coffee beverage preparation, a Olidef-CZ heater and a Chromameter-2 Reflectance Colorimeter, equipped with a CR-300 (Minolta, Osaka, Japan).

Sample pre-treatment

Green coffee (*Coffea arabica* L.) *Mundo Novo*, harvested in 2001/2002, sieve 17/18, fruit without imperfections, were supplied by Ipanema Agro Indústria Ltda. (Alfenas, MG, Brazil). The beans were obtained by two different processes: dry process, which gives natural coffees, and semi-dry process, a modern treatment of the beans which gives peeled coffees. For this study were used beans from dry process. After, they were roasted in a laboratory roaster (1Kg capacity) of two-steps at 200°C, during time sufficient to give "light" roasted samples. The beans were finely ground and packed in impermeable polypropylene/aluminum/polyethylene bags hermetically sealed under vacuum, and stored at –20° C until use. Color analysis of the ground coffee was carried out using a tristimulus colorimeter. The instrument was standardized against a white surface before each measurement. Color was expressed in L*, a*, and b* *Comission Internationale de Eclairage* (CIE) scale parameters.

Sample Extraction

The extraction was done in agreement with Nicoli et al. (6). Initially it was measured 200 mL of water (Mili-Q) and warmed until 90°C. After heating, water was shed on 20g of powder coffee, previously conditioned in Whatman n°3 filter paper, obtaining the aqueous extract, coffee beverage. From the residue remained on the paper and coffee beverage were done the extracts. Then, coffee residue and beverage was submitted to different extraction processes with different liquids extractors in according to Figure 1. After extraction process, extracts were concentrated to 1% with dimethyl-sulfoxide, providing stock solutions.

Free radical scavenging assay

The effect of extracts on $DPPH^{\cdot}$ radical was evaluated in according to the method of Hatano et al. (15). Extracts were diluted to 200ug. mL^{-1}. Then, in 4mL of diluted sample was added 1ml de DPPH 0.0005 mol L^{-1} diluted in

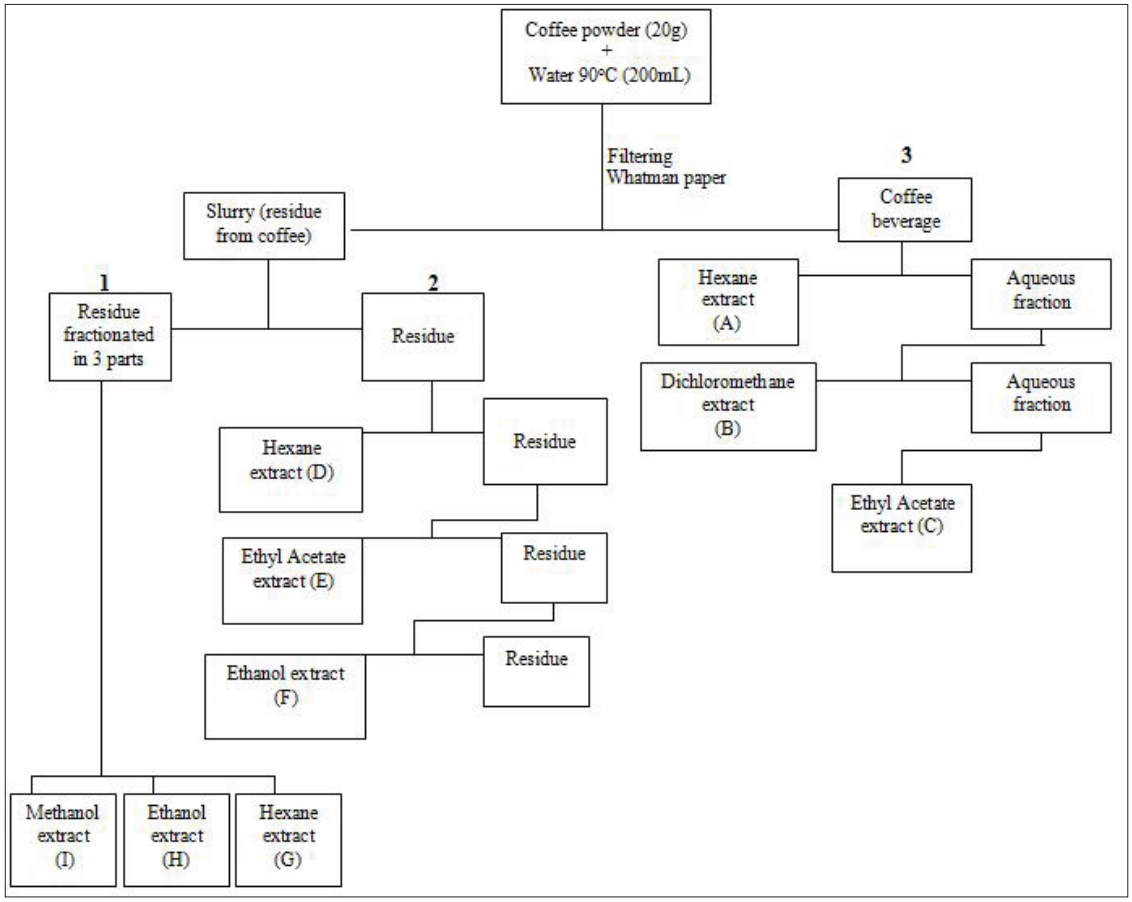

Figure 1: Schematic coffee residue and beverage extraction processes with different solvents. In figure: 1 overnight maceration; 2 soxleth; 3 liquid-liquid partition.

methanol. This mixture was vigorously shaken remained at room temperature for 30 minutes; the absorbance of the mixture was then measured by spectrophotometer at 517 nm. A low absorbance indicates good activity of free radical scavenging. Samples were analyzed in triplicate, taking as standard the activity of ascorbic acid 200ug mL^{-1}, a recognized antioxidant. The activity of free radical scavenging was calculated following the equation:

$$\%AFRS = \frac{Ca - Ta}{Ca} \times 100$$

where %AFRS: percentile activity of free radicals scavenging; Ca: Control absorbance (blank); Ta: Test absorbance (sample).

Reducing power assay

Reducing power of different extracts (50ug. mL^{-1}) was determined according to the method of Oyaizu (16). Samples were mixed with 2.5mL of phosphate buffer (2 M, pH 6.6) and 2.5mL potassic ferricyanide (1%m/v). Then the solution was incubated at 45°C for 20 minutes. After that 2,5mL trichloroacetic acid (10%m/v) was added and the solution was centrifuged at 4000 rpm per minute for 15 minutes. 2.5mL of the supernatant was mixed with 2.5mL of ultra-pure water and 0.5mL ferric chloride (0.1%m/v). The absorbance was measured at 700 nm. The increased in absorbance of the solution indicates an increase in reducing power. Samples were analyzed in triplicate, taking as standard the activity of ascorbic acid 50ug mL^{-1}.

Chelating capacity of Fe^{2+} ion

Capacity of ion chelating Fe^{2+} was conducted in according to the method described by Tang et al. (17). Each sample was diluted with ethanol, resulting in the concentration of 1.0mg mL^{-1}. Then 1mL of sample was transferred to amber tubes. Following were added 3.7mL of ultra-pure water, 0.1mL of $FeSO_4$ 2mM as source of Fe^{2+} and 0.2mL of ferrozine 5mM as chromogenic reagent. The solution was stirred for 20 minutes and analyzed in spectrophotometer at 562nm. The lowest absorbance indicates the best chelating capacity. All tests were performed in triplicate and EDTA 200 mg. mL^{-1}, a recognized chelator, was used as standard. The activity of

EDTA was considered 100% and chelating capacity of the samples was calculated following the equation:

$$\%CC = \frac{Ca - Ta}{Ca} \times 100$$

where %CC: percentile of chelating capacity; Ca: Control absorbance (blank); Ta: Test absorbance (sample).

RESULTS AND DISCUSSION

Free radical scavenging assay

The main mechanism of antioxidants is their interaction with oxidative free radicals such as reactive oxygen and nitrogen species which lead to cancer (18–19). DPPH method works as the following manner: some molecules with antioxidants properties react with a stable free radical, a,a-diphenyl-b-picrylhydrazyl, that has violet color and convert it to a,a-diphenyl-b-picrylhydrazine, with discoloration and decrease absorbance, indicating a free radical scavenging property. DPPH radical has stability, low rate of deterioration and reactivity with the majority of antioxidant compounds such as phenolic compounds and aromatic acids. Only strong reducing agents are able to react with these radicals in a stable system (20). Thus, after extraction with different solvents, free radical scavenging of residue extracts from light roasted beans was evaluated. The highest yields for DPPH radical scavenging were B, C and I, respectively, as showed in Table 1 while acid ascorbic showed 95% activity at the same concentration. In general, antioxidant activity from natural plant extracts is usually attributed to their hydrogen donating ability (20). It is well known that free radicals cause auto-oxidation of unsaturated lipids in food. Antioxidants are believed to intercept the free radical chain of oxidation by donating hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate oxidation of the lipids (21).

Reducing capacity of some compounds may act as a significant indicator of its potential antioxidant activity. The reduction of the ferricyanide complex (Fe^{3+}, yellow) to ferrous form (Fe^{2+}, blue) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm which occurs in the presence of reductants such as antioxidant compounds in the antioxidant samples (22). For the measurements of the reductive ability, Fe^{3+}/Fe^{2+} transformation was investigated in presence of extract using the method of Oyaizu (16).The results of reducing power assay for extracts using potassium ferricyanide reduction method are show in Table 2. For ascorbic acid the reducing power observed was 97.7%. The data presented indicate that reducing power for extracts B, C, E, H and I seem to be the result of their antioxidant activity. The reducing properties are generally associated with the presence of reductones. It is presumed that phenolic compounds may act in similar way as reductones donating electrons and reacting with free radicals converting them to more stable products and terminating the free radical chain reaction (23).

Chelating capacity of Fe^{2+} ion

Fe^{2+} in solution, even if in very low concentration, induces the generation of HO˙ through Fenton's reaction, causing tissue injury and/or cellular death (17). In the evaluation of the chelating activity, the ferrozine, a cromogenic reagent, turns the solution rose with the increase of amount of available Fe^{2+} in solution. Then, lower ions chelation in the sample, larger the number of available ions for reaction with the ferrozine and bigger

Table 2: Reducing power assay of different extracts from coffee beverage (A, B and C) and residue (D, E, F, G, H and I).

Extracts (5 µg mL^{-1})	*Reducing power from Fe^{3+} to Fe^{2+} (%)*
A	0
B	85.4
C	71.6
D	0
E	12.4
F	8
G	0
H	19.3
I	36.2

Table 1: Free radical (DPPH˙) scavenging assay of different extracts from coffee beverage (A, B and C) and residue (D, E, F, G, H and I).

Extracts (200 mg mL^{-1})	*Free radical scavenging (%)*
A	0
B	91.8
C	85.6
D	0
E	0
F	0
G	0
H	0
I	57.4

Table 3: Ion chelating capacity of Fe^{2+} of different extracts from coffee beverage (A, B and C) and residue (D, E, F, G, H and I).

Extracts (5 µg mL^{-1})	*Ion chelating capacity of Fe^{2+} (%)*
A	0
B	4.3
C	9.9
D	0
E	0
F	14.7
G	0
H	0
I	14.8

will be the absorbance. The results presented in the Table 3 showed little chelating activity in relation to the standard, EDTA, which had 99,8% of chelating capacity. The reduced activity probably is due to the decrease of phenolic compounds, chlorogenic acids and even because of the reduced melanoidins formation, through Maillard's reaction, which also have antioxidant activity (17).

CONCLUSION

Coffee is one of the most popular beverages consumed in our planet. This work focused on the antioxidant activity of the some extracts of coffee slurry and beverage to show the potential antioxidant activity of the coffee.

In general, antioxidant activities of extracts were reasonably high due to the presence of polyphenols such as phenolic compounds and chlorogenic acids in a substantial quantity (24). The results reveal that the extracts act as free radical inhibitors and primary antioxidants that interact with free radicals. As showed through $DPPH^{\cdot}$ assay the extracts provide hydrogen donating capabilities to act as antioxidant. We can observe that coffee slurry, although lower than coffee beverage, can be considered as antioxidant and its use can contribute to the chelation and combat to free radicals, which in excess can lead to lipidic peroxidation, with consequent damage to plasmatic membranes and oxidation of desoxirribonucleic acid (18–19).

In comparison with coffee slurry, for scavenging and reduction power assays, coffee beverage presented the best values in the essays, being dichloromethane and ethyl acetate the extractors which showed the best efficiency. Although, considering only chelation capacity assay, the slurry (ethanol and methanol fractions) presented the highest found values. Therefore, we can find a use to this material which is discarded and has a potential antioxidant activity through the extraction and concentration.

ACKNOWLEDGEMENTS

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for the fellowships and Ipanema Agro Indústria Ltda (Alfenas, MG) for providing the coffee samples.

REFERENCES

1. Daglia M., Papetti A., Dacarro C. and Gazzani G. Source Isolation of an antibacterial component from roasted coffee. *Journal of Pharmaceutical and Biomedical Analysis*. **18**: 219–225 (1998).
2. Oliveira J.J.V., Loredo I.S.D., Rodrigues J. and Castro M.F.M. Avaliação dos níveis residuais de clorpirifós, diclorvós e paration metílico na borra e na bebida de café. *Revista Brasileira de Toxicologia*. **15**: 75–78 (2002).
3. Fan L., Pandey A., Mohan R., Soccol C.R. Use of various coffee industry residues for the cultivation of Pleurotus ostreatus in solid state fermentation. *Acta biotechnological*. **20**: 41–52 (2000).
4. Pinelo M., Tress A.G., Pederesen M., Arnous A., Meyer A.S. Effect of cellulases, solvent type, and particle size distribution on the extraction of chlorogenic acid and other phenols from spent coffee grounds, *American Journal Food Technology*. **2**: 641–651 (2007).
5. Silva M.A., Nebra S.A., Silva M.J.M., Sanchez C.G. The use of biomass residues in the Brazilian soluble coffee industry. *Biomass Bioenergy*. **14**: 457–467 (1998).
6. Nicoli M.C., Anese M., Manzocco L., Lerici C.R. Antioxidant properties of coffee brews in relation to the roasting degree. *Lebensmittel-Wissenschaft und-Technologie*. **30**: 292–297 (1997).
7. Daglia M., Cuzzoni M.T., Dacarro C. Antibacterial activity of coffee relationship between biological activity and chemical markers. *Journal Agricultural and Food Chemistry*. **42**: 2273–2274 (1994).
8. Andueza S., Conception C., Nicoli M.C. Comparison of antioxidant and pro-oxidant activity in coffee beverages prepared with conventional and "Torrefacto" coffee. *Lebensmittel-Wissenschaft und Technologie Food Science and Technology*. **37**: 893–897 (2004).
9. Yamaguchi T., Takamura H., Matoba T., Terao J. HPLC method for evaluation of the free radical-scaveging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Bioscience Biotechnology & Biochemistry*. **62**: 1201–1204 (1998).
10. Daglia M., Papetti A., Gregotti C., Berte F., Gazzani G. In vitro antioxidant and ex vivo protective activities of green and roasted coffee. *Journal of Agricultural & Food Chemistry*. **48**: 1449–1454 (2000).
11. Krings U., Berger R.G. Antioxidant activity of some roasted foods. *Food Chemistry*. **72**: 223–229 (2001).
12. Richelle M., Tavazzi I., Offord E. Comparison of the antioxidant activity of commonly consumed polyphenolic beverages (coffee, cocoa and tea) prepared per cup serving. *Journal of Agricultural & Food Chemistry*. **49**: 3438–3442 (2001).
13. Devasagayam T.P.A., Kamat J.P.S., Mohan H., Kasavan P.C. Caffeine as an antioxidant: Inhibition of lipid peroxidation induced by reactive oxygen species. *Biochimica and Biophysica Acta*. **1282**: 63–70 (1996).
14. Dacarro C., Daglia M., Cuzzoni M.T., Bonferoni B. Antibacterial activity of coffee against S.mutans. *Igiene Moderna*. **104**: 379–386 (1994).
15. Hatano T., Kagawa H., Yasuhara T., Okuda T. *Chemistry & Pharmacology Bulletin*. **36**: 1090–1097 (1988).
16. Oyaizu M. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Japan Journal Nutrition*. **44**: 307–315 (1986).
17. Tang S.Z., Kerry J.P., Sheehan D., Buckley D.J. Antioxidative mechanisms of tea catechins in chicken meat systems. *Food Chemistry*. **76**: 45–51 (2002).
18. Loureiro A.P.M., Di Mascio P., Medeiros M.H.G. Formação de Adutos Exocíclicos com Bases de DNA: Implicações em Mutagênese e Carcinogênese. *Química Nova*. **25**: 777–793 (2002).
19. Willcox J.K., Ash S.L., Catignani G.L. Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutritional*. **44**: 275–295 (2004).
20. Yokozawa T., Chen C.P., Dong E., Tanaka T., Nonaka G.I., Nishioka I. Study of the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl picrylhydrazyl radical *Biochemical Pharmacoly*. **56**: 213–222 (1998).
21. Aruoma O.I., Halliwell B. *Free radicals and food additives*, (Taylor and Francis Ltd, London, 1991) 17–35.
22. Chung Y., Chang C., Chao W., LIN C., Chou S. Antioxidative activity and safety of the 50% ethanolic extract from red bean fermented by *Bacillus subtilis* IMR-NK1. *Journal of Agricultural and Food Chemistry*. **50**: 2454–2458 (2002).
23. Duh P. D. Antioxidant activity of budrock (Arctium lappa L.): Its scavenging effect on free radical and active oxygen. *Journal of American Oil Chemists Society*. **75**: 455–461 (1998).
24. Duarte S.M.S., Abreu C.M.P., Menezes H.C., Santos M.H., Gouvea C.M.C.P. Effect of processing and roasting on the antioxidant activity of coffee brews. *Ciência e Tecnologia de Alimentos*. **25**: 387–393 (2005).