Food Research International 45 (2012) 197–203

Contents lists available at SciVerse ScienceDirect

Food Research International

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

In vitro prospective effects of various traditional herbal coffees consumed in Anatolia linked to neurodegeneration

Nazim Sekeroglu [a], F. Sezer Senol [b], Ilkay Erdogan Orhan [b,c,*], A. Rifat Gulpinar [d], Murat Kartal [d], Bilge Sener [b]

[a] Department of Biology, Faculty of Art and Sciences, Kilis 7, Aralik University, 79000 Kilis, Turkey
[b] Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey
[c] Pharmacognosy and Pharmaceutical Botany Unit, Faculty of Pharmacy, Eastern Mediterranean University, GaziMagosa, North Cyprus, via Mersin10, Turkey
[d] Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, 06100 Ankara, Turkey

ARTICLE INFO

Article history:
Received 24 August 2011
Accepted 5 October 2011

Keywords:
Traditional coffees
Cholinesterase inhibition
Antioxidant activity
Fatty oil

ABSTRACT

Various herbal coffee varieties are consumed traditionally in Turkey. In the current study, the ethanol extracts of the coffee and seed samples obtained from *Gundelia tournefortii* (tumble thistle), *Nigella sativa* (black cumin), *Phoenix dactylifera* (date), and *Ceratonia siliqua* (carob) as well as a sample of instant coffee (Nescafe®, green blend) were tested against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and tyrosinase, the enzymes connected to neurodegeneration. Since oxidative stress is strongly associated with neurodegeneration, antioxidant activity of the extracts was also determined. Antioxidant activity of the extracts was measured using anti-radical and metal-related methods. Total phenol and flavonoid contents were calculated using Folin–Ciocalteau's and $AlCl_3$ reagents, respectively. Fatty acid compositions of the seed oils of tumble thistle and black seed were analyzed by GC–MS. Our results showed that the date sample exerted the highest AChE and BChE inhibition at 300 μg mL^{-1} (52.96% and 83.22%, respectively).

1. Introduction

Drinking of coffee is an important part of Turkish culture, one of the valuable traditions of daily life, and a way of socializing since centuries. Turkish coffee was first introduced during the Ottoman Empire in 1543 and people around the world have heard of the fame of Turkish coffee (Birsel, 1991). It is also a symbol of Turkish hospitality to offer coffee to the guests. Although the Turkish coffee prepared from the seeds of *Coffea arabica* L. (Rubiaceae) is the most famous, there are some other types of herbal coffee traditionally consumed in Turkey. Among them, "kenger coffee" obtained from *Gundelia tournefortii* L. (GT, tumble thistle, tumbleweed, Asteraceae), "çörek otu coffee" from *Nigella sativa* L. (NS, black cumin, Ranunculaceae), "hurma coffee" from *Phoenix dactylifera* L. (PD, date, Arecaceae), and "keçiboynuzu coffee" from *Ceratonia siliqua* L. (CS, carob, Fabaceae) are some of the herbal coffees consumed in Turkey. Although *P. dactylifera* is not grown naturally in Turkey and usually obtained through import from Arab countries, this plant has been recorded to be used for memory enhancing purpose traditionally in our country (I.E.O. — personal communication). The above-mentioned coffees are obtained in the powdered form after roasting and grinding of their seeds, which gives extra flavor and aroma. Making style of these coffees is similar to that of the Turkish coffee, which is explained as follows. The powdered crude coffees are measured in a certain amount, mixed with water in a special coffee pot, stirred occasionally, and let it boil slowly until a foam is occurred on the top, which takes about a few minutes.

Neurodegeneration is a complex and multifactorial procedure in human brain and, unfortunately, the prevalence of neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) is increasing. On the other hand, effective treatments and medications are still absent to seize the disease. For AD, the most applied medication class is cholinesterase inhibitors (Orhan, Orhan, Subutay-Oztekin, Ak, & Sener, 2009). Oxidized dopamine metabolites having a crucial function in the degeneration of nigrostriatal dopaminergic neurons in PD is induced by tyrosinase (TYRO) through its oxidase activity and, therefore, inhibition of TYRO is important in prevention of PD (Hasegawa, 2010). Another factor contributing to pathology of neurodegenerative diseases is oxidative stress, which leads to neuronal death (Halliwell, 2006).

In view of the fact that the aforementioned coffees are consumed commonly in Anatolia, especially in the southern part, and folkloric use of date is recorded for memory-improvement, we decided to investigate the ethanol extracts of the aforementioned coffee samples along with their source seeds for their potential effects in neurodegeneration *via* enzyme inhibition against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), and tyrosinase (TYRO) using ELISA microplate reader. In addition, a sample of commercial instant coffee brand (Nescafe®, green blend, GB) was also tested with other four traditional coffee varieties for a comparative purpose. Antioxidant activity of the extracts was measured using radical scavenging activity tests and metal-related tests including metal-

* Corresponding author at: Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330 Ankara, Turkey. Tel.: +90 312 2023186; fax: +90 312 2235018.
E-mail addresses: iorhan@gazi.edu.tr, ilkay.orhan@emu.edu.tr (I.E. Orhan).

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.10.008

chelation capacity, ferric-reducing antioxidant power (FRAP), and phosphomolybdenum reducing power (PRAP). Total phenol and flavonoid contents in the extracts were calculated using Folin–Ciocalteau and $AlCl_3$ reagents, respectively. The fatty oils obtained from the seeds of the tumble thistle and black cumin samples were analyzed for their fatty acids by gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

2.1. Coffee and seed materials

The coffee and seed samples of *G. tournefortii* (GT, tumble thistle), *N. sativa* (NS, black cumin), *P. dactylifera* (PD, date), and *C. siliqua* (CS, carob) were kindly provided by Sekeroglu Baharatcilik Company in Kilis province (Turkey) in 2011. Instant coffee brand (Nescafe®, green blend, GB) was purchased from a supermarket in Ankara province (Turkey), in 2011.

2.2. Extraction procedure

The seed samples were ground by a mechanic grinder and weighed precisely in a digital balance (Shimadzu). Then, each coffee variety already in powdered form was also weighed accurately and the coffee and seed samples were extracted with ethanol (80%) during 3 days at room temperature by shaking occasionally. Since the solubility of the powdered seeds and coffee samples was not good in water at all, the extracts were prepared with ethanol. The ethanolic phases were filtered and evaporated *in vacuo* until dryness to give the crude extracts. Percentage yields of the ethanol extracts (w/w) are given as follows; GT-seed: 0.97, GT-coffee: 6.44, NS-seed: 2.35, NS-coffee: 4.33, CS-seed: 4.88, CS-coffee: 9.64, PD-seed: 2.65, PD-coffee: 3.12, GB-coffee: 14.74.

The seed samples belonging to four plant species were separately subjected to continuous extraction technique using Soxhlet apparatus, which was extracted with *n*-hexane for 8 h. Then, organic phases were filtrated until dryness to give the fatty oils. Percentage yields of the fatty oils (w/w) are given as follows; GT: 5.93, NS: 7.31, PD: 0.06, CS: 0.09.

2.3. Determination of total phenol and flavonoid contents in the extracts

Phenolic compounds were determined in accordance with Folin–Ciocalteau's method (Singleton & Rossi, 1965). In brief, a number of dilutions of gallic acid were obtained to prepare a calibration curve. The extracts and gallic acid dilutions were mixed with 750 μL of Folin–Ciocalteau's reagent and 600 μL of sodium carbonate in test tubes. The tubes were then vortexed and incubated at 40 °C for 30 min. Afterward, absorption was measured at 760 nm at a Unico 4802 UV–visible double beam spectrophotometer (USA). Total flavonoid content of the extracts was calculated by aluminum chloride colorimetric method (Woisky & Salatino, 1998). To sum up, a number of dilutions of quercetin were obtained to prepare a calibration curve. Then, the extracts and quercetin dilutions were mixed with 95% ethanol, aluminum chloride reagent, 0.1 mL of sodium acetate as well as distilled water. Following incubation for 30 min at room temperature, absorbance of the reaction mixtures was measured at wavelength of 415 nm with a Unico 4802 UV–visible double beam spectrophotometer (USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g^{-1} extract), respectively.

2.4. AChE and BChE inhibitory activity assays

AChE and BChE inhibitory activity was measured by slightly modified spectrophotometric method of Ellman, Courtney, Andres, and Featherstone (1961). Electric eel AChE (Type-VI-S, EC 3.1.1.7, Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction, respectively. 5,5′-Dithio-bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents and conditions were same as described in our previous publication (Senol et al., 2010). Hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (VersaMax Molecular Devices, USA). The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software. Percentage of inhibition of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank (ethanol in phosphate buffer pH = 8) using the formula $(E-S)/E \times 100$, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galanthamine (Sigma, St. Louis, MO, USA) was used as the reference.

2.5. Tyrosinase inhibitory activity assay

Inhibition of tyrosinase (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) was determined using the modified dopachrome method with L-DOPA as substrate (Masuda, Yamashita, Takeda, & Yonemori, 2005). Assays were conducted in a 96-well microplate using ELISA microplate reader (VersaMax Molecular Devices, USA) to measure absorbance at 475 nm. An aliquot of the extracts dissolved in DMSO with 80 μL of phosphate buffer (pH 6.8), 40 μL of tyrosinase, and 40 μL of L-DOPA were put in each well. Results were compared with control (DMSO). Baicalein (Sigma, St. Louis, MO, USA) was used as the reference. The percentage tyrosinase inhibition (I%) was calculated as follows:

$$I\% = \left(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}\right)/\text{Absorbance}_{\text{control}} \times 100.$$

2.6. Antioxidant activity by radical-formation methods

2.6.1. DPPH radical scavenging activity

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined by the method of Blois (1958). The samples and references dissolved in methanol (75%) were mixed with DPPH solution (1.5×10^{-4} M). Remaining DPPH amount was measured at 520 nm using a Unico 4802 UV–visible double beam spectrophotometer (USA). Gallic acid was employed as the reference. Inhibition of DPPH in percent (I%) was calculated as given below:

$$I\% = \left[\left(A_{\text{blank}} - A_{\text{sample}}\right)/A_{\text{blank}}\right] \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample), and A_{sample} is the absorbance of the extracts/reference. Analyses were run in triplicate and the results were expressed as average values with S.E.M. (Standard error mean).

2.6.2. DMPD radical scavenging activity

Principal of the assay is based on reduction of the purple-colored radical $DMPD^{+}$ (*N*,*N*-dimethyl-*p*-phenylenediamine) (Schlesier, Harvat, Bohm, & Bitsch, 2002). According to the method, a reagent comprising of 100 mM DMPD, 0.1 M acetate buffer (pH = 5.25), and 0.05 M ferric chloride solution, which led to formation of DMPD radical, was freshly prepared and the reagent was equilibrated to an absorbance of 0.900 ± 0.100 at 505 nm. Then, the reagent was mixed up with 50 μL of the extract dilutions and absorbance was taken at