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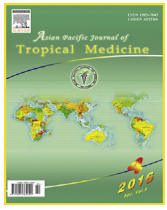

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Antioxidant potential, tannin and polyphenol contents of seed and pericarp of three *Coffea* species

Éva Brigitta Patay[1], Nikolett Sali[2,3], Tamás Kőszegi[2,3], Rita Csepregi[1], Viktória Lilla Balázs[1], Tibor Sebastian Németh[4], Tibor Németh[4], Nóra Papp[1*]

[1]*Institute of Pharmacognosy, Medical School, University of Pécs, Rókus 2, Pécs, 7624, Hungary*

[2]*Department of Laboratory Medicine, Medical School, University of Pécs, Ifjúság 13, Pécs, 7624, Hungary*

[3]*János Szentágothai Research Center, Ifjúság 20, Pécs, 7624, Hungary*

[4]*Department of Pharmacognosy, Faculty of Medicine and Pharmacy, University of Oradea, Piaţa 1 Decembrie u. 10, Oradea, 410073, Romania*

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ABSTRACT

Objective: To investigate the antioxidant activity, total phenolic and total tannin content of the pericarp and the seed of *Coffea benghalensis* (*C. benghalensis)* and *Coffea liberica* compared to *Coffea arabica* (*C. arabica*).

Methods: The antioxidant potential, total tannin and polyphenol contents of the immature and mature seed and pericarp of *C. benghalensis* and *Coffea liberica* were quantified and compared to *C. arabica*. Enhanced chemiluminescence (ECL), 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity, Folin-Ciocalteau method and total tannin content assays were used.

Results: Trolox equivalent (TE/g plant material) values obtained by ECL and DPPH methods showed loose correlation (r^2 = 0.587) while those measured by oxygen radical absorbance capacity assay were higher without correlation in each plant. A closer correlation was detected between the ECL method and the percentage antioxidant activity of the DPPH technique (r^2 = 0.610 7) in each species, however the immature pericarp of *C. benghalensis* showed much higher DPPH scavenging potential than was seen in the ECL assay. The immature pericarp of *C. benghalensis* expressed the highest tannin and polyphenol content, and a high polyphenol level was also detected in the immature seed of *C. arabica*. The immature pericarp of Bengal and Liberian coffees showed the largest amount of phenolic contents.

Conclusions: The obtained data highlight the potential role of *C. benghalensis* as a new source of natural antioxidants and polyphenols compared to *C. arabica*.

1. Introduction

Coffee (*Coffea*) species are evergreen shrubs or small trees which are native to the Ethiopian mountains [1,2]. They belong to the Rubiaceae family, which is the largest plant family in the world involving 450 genus and 6500 species [3,4]. Nowadays, more than 120 *Coffea* species and their varieties are mentioned in scientific reports [5,6]. They grow in the tropical and subtropical areas, especially in the Equatorial region at an altitude of (200–1 200) m and between (18–22) °C [7]. *Coffea arabica* (*C. arabica*) L., *Coffea robusta* (*C. robusta*) L. Linden, and *Coffea liberica* (*C. liberica*) Hiern have significant commercial value, occupying the second place after petrol on the international market [1,2]. Coffee is one of the most widely consumed beverages worldwide, with an annual consumption rate of approximately 7 million tons according to FAO [8].

C. arabica (Arabic coffee) originated from Ethiopia is the most widespread coffee species. It provides 80% of the coffee production of the world [9]. The wild species *Coffea benghalensis* (*C. benghalensis)* Roxb. (Bengal coffee), which has been reclassified into the *Psilanthus* genus *(Psilanthus benghalensis* Roxb. Ex Schult.), is a small shrub in South and

*Corresponding author: Nóra Papp, Institute of Pharmacognosy, Medical School, University of Pécs, Rókus 2, Pécs, 7624, Hungary.
Tel: +36 72503650x28824
E-mail: nora4595@gamma.ttk.pte.hu

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Southeast Asia. Although, Bengal coffee is rarely used in the industry, the cafesterol and bengalensol content, as well as the antimicrobial and antioxidant effects of the fruit have been determined [10–12]. *C. liberica* (Liberian coffee) is also native to Africa, and it provides 2% of the total coffee production of the world. Despite its lower demand, the volatile extract of its immature beans possessed higher antioxidant capacity than that of *C. arabica* and *C. robusta* [13].

In oxidative stress, reactive oxygen species have been suggested to participate in the initiation and propagation of chronic diseases such as cardiovascular and inflammatory diseases, cancer, and diabetes [14]. Antioxidants which are found naturally in many foods and beverages provide health benefits in preventing heart disease and cancer by fighting against cellular damage caused by free radicals in the body [15]. Coffee species are rich in biologically active substances and polyphenols such as chlorogenic acid, ferulic acid, caffeic acid, sinapic acid, kaempherol, quercetin, nicotinic acid, trigonelline, quinolic acid, tannic acid, pyrogallic acid and caffeine which possess antioxidant, hepatoprotective, antibacterial, antiviral, anti-inflammatory and hypolipidemic effects [16–24]. These compounds play an important role against pathogens and abiotic stress such as changes in temperature, water content, exposure to UV light levels and deficiency in mineral nutrients [25]. In coffee species, chlorogenic acid content and its *cis*-isomers have been determined to be higher in the leaves than the seeds which prove the effect of UV radiation on the geometric isomerisation of chlorogenic acid in the leaves [26]. The local use of coffee extracts could prevent various dermatological disorders, in addition, they also could have UV protection for skin. During a clinical study 30 patients having dermatological face problems were locally treated with coffee seed extract. In comparison with the standard creams with placebo effect, coffee extract reduced wrinkles and pigmentation, as well as it improved the appearance of patients' skin [27]. The silverskin of *C. arabica* and *C. robusta* have antioxidant activity [28], while the extract of the green seed has an anti-inflammatory effect [29]. The regular consumption of coffee reduce the kidney, liver, premenopausal breast and colon cancer [30].

Although more than 100 *Coffea* species are known nowadays, only a few taxa have been extensively analyzed at present. Based on the widely used enhanced chemiluminescence (ECL), 2,2-diphenyl-1-picrylhydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) assays, total phenolic, and tannin methods [31], the aim of this study was to investigate the antioxidant activity, total phenolic and total tannin content of the pericarp and seed of *C. benghalensis* and *C. liberica* compared to the thoroughly studied *C. arabica*. The analyses were carried out to find new sources of natural antioxidants for nutraceuticals, and a new utilization of wasted residues of coffee products.

2. Material and methods

2.1. Plant materials

The mature and immature fruits of *C. benghalensis*, *C. liberica*, and *C. arabica* were collected in the Botanical Garden of the University of Pécs in the spring of 2014. The samples were air-dried at room temperature in the shade. Voucher specimens were deposited and labeled with unique codes at the Institute of Pharmacognosy, University of Pécs. For the antioxidant assays, samples were ground (0.25 g each) and extracted with 5 mL 50% ethanol (Merck). The extracts were shaken for 20 min (Edmund Bühler, Labortechnik-Materialtech, Johanno Otto GmbH), then filtered and stored at 4 °C in the dark until analyses (less than 7 d).

2.2. Chemicals and reagents

All chemicals, used for antioxidant assays, were of analytical or spectroscopic grade purity and highly purified water (<1 μS) was used in our experiments. Horseradish peroxidase (POD from Sigma-Aldrich), 1 mg/mL bovine serum albumin (BSA, Serva) in 50 mM phosphate buffer pH 7.4, H_2O_2 (Molar Chemicals) diluted with citric acid (Ph. Hg. Eur), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), luminol, para-iodophenol, diphenyl-2,2-picryl-hydrazyl (DPPH stable free radical), fluorescein-Na_2 salt, 2,2′-azobis (2-methylpropionamidine) dihydrochloride (AAPH, all from Sigma-Aldrich), methanol and ethanol (Reanal, Hungary) were used as received. In the ORAC assay 75 mM phosphate buffer of pH 7.4 was applied.

Chemicals used for Folin-Ciocalteau methodology and for measurement of total tannin were the followings: $AlCl_3$ (Alfa Aesar), acetone, 25% HCl, ethyl acetate, 5% methanol-acetic acid (Molar Chemicals), distilled water, solution of sodium carbonate (Lach-Ner), phosphor-molybdo-tungstic reagent (Sigma-Aldrich), holystone (Reanal), hide powder, hexamethylene tetramine, and pyrogallol (VWR).

2.3. DPPH assay

Four mg DPPH in 100 mL methanol (0.1 mmol/L) was prepared and kept in the fridge being stable for at least 1 wk. For absorbance measurements standard 96-well microplates (Sarstedt) were applied. Twenty μL Trolox/blank/sample and 180 μL DPPH solution were pipetted into the wells (using a multichannel pipette), mixed and the absorbance was read at 517 nm after 30 min incubation in the dark at 25 °C [32–34].

2.4. ECL

We adapted and modified the method of Muller *et al* [35] as follows.

Reagents: Before the analysis 15 μU/mL POD working solution was freshly prepared from 1.5 U/mL POD stock stored at −20 °C in phosphate buffered saline (PBS, pH 7.4) by dilution with the BSA containing phosphate buffer and was kept on ice. A working reagent of 1360 μM H_2O_2 was also freshly diluted with 0.1% citric acid from 10 M concentrated stock solution and was also kept on ice, protected from light. During the whole period of measurements these reagents were stored in melting ice. Both working solutions were stable for at least several hours.

The chemiluminescence detection reagent was prepared separately by dissolving luminol and p-iodophenol in 0.2 M boric acid/NaOH buffer, pH 9.6 and was refrigerated at 4 °C in brown bottles with a shelf life of several weeks. Trolox was used as standard in both assays. Trolox at 1 mM concentration was dissolved freshly in 50% ethanol weekly and kept at 4 °C.