Gamma irradiation improves the extractability of phenolic compounds in *Ginkgo biloba* L.

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ABSTRACT

Irradiation has been increasingly recognized as an effective decontamination technique that ensures the chemical and organoleptic quality of medicinal and aromatic plants. The aim of the present study was to evaluate the effects of gamma irradiation in the phenolic compounds of *Ginkgo biloba* L. (infusion and methanol/water extract), widely used in traditional medicine and in dietary supplements. Twenty-five compounds were detected, eighteen of which were flavonoids, one phenolic acid, five terpene lactones and one unknown compound. Among the quantified phenolic compounds, flavonoids were the main group present, being two kaempferol derivatives the major compounds found: kaempferol-3-*O*-dirhamnosylglucoside and kaempferol-3-*O*-rutinoside. The irradiation with the highest dose (10 kGy) is sufficient to guarantee the product disinfestation and microbial decontamination, also contributing to an increase in the extractability of phenolic compounds, both in methanol/water and infusion preparations.

Keywords: *Ginkgo biloba*; Phenolic compounds; Gamma irradiation.

1. Introduction

The interest for natural antioxidants has been increasing over the years. Phenolic compounds comprise a very large group of biologically active molecules, being appreciated for their beneficial effects on health (physiologically active compounds with anti-allergic, anti-atherogenic, antimicrobial, antithrombotic, anti-inflammatory, antioxidant, cardioprotective and vasodilatory effects) (Mendel and Youdim, 2004; Balasundram et al., 2006; Martins et al., 2011). Their mechanism of action as antioxidants is considered essential regarding the reduction of the oxidation processes in the body, playing an important role in maintaining health, including protection of the cells and biomacromolecules and, therefore, intervening against certain human diseases (cancer, inflammatory diseases, neurological degeneration, heart disease, and many others) (Lan et al., 2007; Rawat et al., 2011; Acosta-Estrada et al., 2014). Thus, the most cited forms of intervention of antioxidant potential of phenolic compounds are their ability to scavenge reactive oxygen species and to chelate metal ions (Port's et al., 2013).

Plants are some of the most important sources of natural antioxidants including phenolic compounds (*e.g.*, phenolic acids and flavonoids, phenolic diterpenes and tannins), which have been related with the bioactivity of several medicinal plants (Sati et al., 2013). One of those plants that has been highly studied is *Ginkgo biloba* L., due to its use in traditional medicine, but also by professionals in the medical field in order to treat problems typically associated with aging, such as intermittent claudication, decreased mental vitality in old age (mental confusion, memory loss, dementia praecox, concentration problems), poor circulation and tinnitus (Diamond et al., 2000). The extracts from *G. biloba*, such as EGb761 in Tebonin®, are also used as alternative therapy against Alzheimer's disease (van Beek and Montoro, 2009; Parimoo et al.,

2014). The products with this plant are commercially available in various forms: leaves for infusions preparation, standardized extracts, pills, capsules or oral solutions (Liu et al., 2014).

The effectiveness of the therapeutic use of this plant leads to a strong demand from the pharmaceutical industry (Koch, 2005). However, due to the strict hygiene standards applied for raw materials to be incorporated into pharmaceuticals and/or dietary supplements, efficient decontamination methods are necessary, avoiding other alternatives that may leave chemical residues in the plant (Haleem et al., 2014).

The use of irradiation has been increasingly accepted for decontamination and conservation, since it does not significantly affect (at specific doses) the organoleptic and physico-chemical properties of the irradiated matrices (Alothman et al., 2009; Kirkin et al., 2014), including *G. biloba* (Pereira et al., 2015). This method reduces reliance on chemical fumigants (ethylene oxide and methyl bromide), which have been pointed out as mutagens and carcinogens to humans, leave chemical residue on plants and destroy the ozone layer in the atmosphere (Migdal & Owczarczyk, 1998; Chmielewski & Migdal, 2005).

Therefore, this study aims to evaluate if gamma irradiation (at doses of 1 and 10 kGy) improves the extraction of phenolic compounds using *Ginkgo biloba* (infusion and methanolic extract) as source material.

2. Materials and methods

2.1. Samples and samples irradiation

Ginkgo biloba L. samples were supplied by Américo Duarte Paixão Lda., Alcanede (Portugal), imported from China, as dry leaves material. The botanical identification was confirmed by the biologist, Dr. Carlos Aguiar of the Escola Superior Agrária of the

Polytechnic Institute of Bragança (Trás-os-Montes, Portugal). The samples were divided into three groups: control (non-irradiated, 0 kGy), group 1 and group 2, where 1 kGy and 10 kGy were, respectively, the predicted doses.

The irradiation was performed in a Co-60 experimental chamber (Precisa 22, Graviner Manufacturing Company Ltd., UK) with total activity 177 TBq (4.78 kCi), in September 2013, and the estimated dose rate for the irradiation position was obtained with Fricke dosimeter. During irradiation process, the dose was estimated using Amber Perspex routine dosimeters (batch V, from Harwell Company, U.K.), following the procedure previously described by Pereira et al. (2015). The estimated doses, dose rates and dose uniformity ratios (D_{max}/D_{min}) were, respectively: 1.20 ± 0.07 kGy, 2.57 ± 0.15 kGy h^{-1}, 1.20 for sample 1 and 8.93 ± 0.14 kGy, 1.91 ± 0.03 kGy h^{-1}, 1.02 for sample 2. For simplicity, in the text and tables we considered the values 0, 1 and 10 kGy, for the doses of non-irradiated and irradiated groups 1 and 2, respectively.

After irradiation, the samples were reduced to powder and mixed to obtain homogenized samples for subsequent analysis.

2.2. Standards and Reagents

For irradiation: To estimate the dose and dose rate of irradiation a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM, 1992) and Amber Perspex dosimeters (batch V, from Harwell Company, UK) were used. The acid aqueous Fricke dosimeter solution was prepared using ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, USA).

For chemical analyses: HPLC-grade acetonitrile was obtained from Merck KgaA (Darmstadt, Germany). Formic acid was purchased from Prolabo (VWR International, Fontenay-sous-Bois, France). Phenolic standards were from Extrasynthèse (Genay, France). Water was treated in Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

2.3. Phenolic compounds

2.3.1. Extraction procedure

Methanol/water extracts: each sample (1 g) was extracted with 30 mL of methanol/water 80:20 (v/v) at room temperature, 150 rpm, for 1 h. The extract was filtered through Whatman 4 paper. The residue was then re-extracted twice, with additional 30 mL portions of methanol/water 80:20 (*v/v*). The combined extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210), until complete removal of methanol. The aqueous phase was lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) (Barros et al., 2013).

Infusions preparation: each sample (1 g) was added to 200 mL of boiling distilled water and left to stand at room temperature for 5 min, and then filtered under reduced pressure; afterwards the obtained infusion was frozen and lyophilized (Barros et al., 2013).

Methanol/water extracts and lyophilized infusions were re-dissolved in 20% aqueous methanol and water, respectively, at 20 mg/mL and filtered through a 0.22 µm disposable LC filter disc for High Performance Liquid Chromatography (HPLC) analysis.

2.3.2. Analysis of phenolic compounds

Phenolic compounds were determined by HPLC (Hewlett-Packard 1100, Agilent Technologies, Santa Clara, CA, USA) as previously described by the authors (Barros et al., 2013). Double online detection was carried out in a DAD using 280 nm and 370 nm as preferred wavelengths and in a mass spectrometer (API 3200 Qtrap, Applied Biosystems, Darmstadt, Germany) connected to the HPLC system via the DAD cell outlet. The phenolic compounds were characterized according to their UV and mass spectra and retention times, and comparison with authentic standards when available. The phenolic compounds were identified by comparing their retention time, UV–vis and mass spectra with those obtained from standard solutions, when available. Otherwise, peaks were tentatively identified comparing the obtained information with available data reported in the literature. For quantitative analysis, a calibration curve for each available phenolic standard was constructed based on the UV signal. For the identified phenolic compounds for which a commercial standard was not available, the quantification was performed through the calibration curve of other compounds from the same phenolic group. The results were expressed in mg/g of methanol/water extract and lyophilized infusion.

2.4. Statistical analysis

Three samples from each group were analysed and all the assays were carried out in triplicate. The results are expressed as mean values±standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD Test with $\alpha = 0.05$. This treatment was carried out using SPSS v. 22.0 program (IBM Corp.).

3. Results and Discussion

3. Results and Discussion

The chromatographic profile of non-irradiated and irradiated at 10 kGy *G. biloba* samples, obtained after methanol/water extraction, and recorded at 370 nm is shown in **Figure 1**; compound characteristics and tentative identities are presented in **Table 1**. Twenty-five compounds were detected, eighteen of which were flavonoids, one phenolic acid, five terpene lactones and one unknown compound.

Protocatechuic acid (compound **3**), myricetin-3-*O*-rutinoside (compound **11**), quercetin-3-*O*-rutinoside (compound **15**), quercetin-3-*O*-glucoside (compound **17**), kaempferol-3-*O*-rutinoside (compound **19**), isorhamnetin-3-*O*-rutinoside (compound **20**) and isorhamnetin-3-*O*-glucoside (compound **22**) were positively identified according to their retention, mass and UV-vis characteristics by comparison with commercial standards. All the compounds mentioned above, with the exception of protocatechuic acid were previously reported in leaves of *G. biloba* (Tang et al., 2001; Zhang et al., 2007; Ding et al., 2008; Lin et al., 2008).

Peaks 1, 2, 4-6 and 12 were associated to terpene trilactones. That type of compounds have low UV absorption and coexisting substances present in the complex matrix of *G. biloba* extracts make it difficult for their detection and quantification, using UV detection (Sloley et al., 2003; Mesbah et al., 2005). Nonetheless, they could be detected in the analysed extracts and tentatively identified based on their MS characteristics and comparison with data reported in the bibliography (Bedir et al., 2002; van Beek, 2002; Ding et al., 2008; Lin et al., 2008; van Beek and Montoro, 2009; Rossi et al., 2011; Liu et al., 2014); in particular, bilobalide (compound **4**), ginkgolide A (compound **5**), ginkgolide B (compound **6**) and ginkgolide C derivative (compound **12**). The latter compound should correspond to a derivative of ginkgolide C, since ginkgolide C would be expected to elute earlier than ginkgolide A and B (Ding et al., 2008; Lin et al., 2008;

van Beek & Montoro, 2009). No identification could be assigned to compound **1** ([M−H]$^-$ at *m/z* 453), although the major *m/z* fragment at 407 might point to it was a derivative of ginkgolide A. No conclusions could be drawn about the identity and nature of compound **2** ([M-H]$^-$ at *m/z* 449). Three compounds with the same pseudomolecular ion were also detected by Ding et al. (2008) in *G. biloba* supplements, which were assigned as unknown glycosyl flavonoids. However, the flavonoid nature of the compound was not clear in our samples, but the presence of MS^2 fragments at *m/z* 179 and 113 also observed in compounds 1 and 6 rather suggested that it could be a ginkgolide, which remained unknown.

The rest of compounds showed UV and mass characteristics coherent with flavonol glycosides. Compounds **9, 19** and **23** ([M-H]$^-$ at *m/z* 593) presented characteristics that match a structure of kaempferol bearing deoxyhexosyl and hexosyl residues. Ding et al. (2008) also detected three compounds with the same mass in *G. biloba* supplements, all of them identified as kaempferol-*O*-rhamnosyl-glucoside. Zhang et al. (2007) and Lin et al. (2008) also found two compounds with similar characteristics in *G. biloba* leaves, one of them kaempferol-3-*O*-rutinoside (positively identified as peak **19** in our samples) and the other one as kaempferol-3-*O*-glucosyl-(1,2)-rhamnoside. This latter identity was assumed for peak **23** owing to its delayed elution in relation to peak 19, as reported by those authors. As no further information was available for compound **9** it was just assigned as a kaempferol-*O*-rhamnosyl-glucoside. Similar reasoning was applied for the identification of compounds **15** and **18** showing the same pseudomolecular ion [M-H]$^-$ at *m/z* 609, which were respectively identified as quercetin-3-*O*-rutinoside and quercetin-3-*O*-glucosyl-(1,2)-rhamnoside, as also reported Zhang et al. (2007) and Lin et al. (2008).

Compounds **7, 10** and **24** presented the same pseudomolecular ion $[M-H]^-$ at *m/z* 755. Their MS^2 spectra pointed to they are derived from different aglycones, i.e., kaempferol (**7**) and quercetin (**10** and **24**). Different compounds with similar UV and mass characteristics were reported by Lin et al. (2008) and Ding et al. (2008) in *G. biloba* leaves and supplements. Compound **7** would correspond to a kaempferol derivative bearing one deoxyhexosyl and two hexosyl residues; the observation of a main MS^2 fragment at *m/z* 593 from the lost of the hexosyl residue suggested that this latter was located at a different position of the other two glycosyl moieties that could be constituting a disaccharide. Although no information about the actual nature and position of the sugar substituents can be obtained from the available data, based on the previous comments assumption and the identification made by Lin et al. (2008) the compound was tentatively assigned as kaempferol-3-*O*-rhamnosylhexoside-7-*O*-glucoside. Characteristics of compound **10** were consistent with a quercetin derivative possessing two deoxyhexosyl and one hexosyl residues. The fact that only one MS^2 fragment was released corresponding to the aglycone (i.e., *m/z* at 301, quercetin) would suggest that the three sugars constituted a trisaccharide; based on this assumption the compound was tentatively identified as quercetin 3-*O*-2’’,6’’-dirhamnosylglucoside reported in *G. biloba* leaves by Lin et al. (2008). Compound **24** showed different UV and MS^2 spectra than compound **10**, presenting maximum wavelength at 316 nm and an additional minor MS^2 fragment at *m/z* 609 (loss of 146 mu that may correspond to either a rhamnosyl or a *p*-coumaroyl moiety), due to its delayed retention time and previous identifications of similar compounds by Lin et al. (2008) and Ding et al. (2008), this compound was tentatively assigned as quercetin-3-*O*-*p*-coumaroyl-rhamnosylhexoside. Similar reasoning was applied for assigning compounds **13** and **25,** both showing pseudomolecular ions $[M-H]^-$ at *m/z* 739, which were tentatively identified respectively

as kaempferol-3-*O*-dirhamnosylglucoside and kaempferol-3-*O*-*p*-coumaroyl-rhamnosylhexoside, as also proposed by Lin et al. (2008).

Mass spectra characteristics of compounds **8** ($[M-H]^-$ at *m/z* 785) and **14** ($[M-H]^-$ at *m/z* 769) were similar to those of compounds 7 and 13 respectively, but derived from an isorhamnetin aglycone as revealed by the MS^2 fragment produced at *m/z* 315. Based on this observation and previous identifications by Lin et al. (2008), these compounds were respectively assigned as isorhamnetin-3-*O*-rhamnosylhexoside-7-*O*-glucoside and isorhamnetin-3-*O*-dirhamnosylglucoside. Compounds **16** ($[M-H]^-$ at *m/z* 639) and **21** ($[M-H]^-$ at *m/z* 447) were assigned as patuletin-3-*O*-rutinoside and quercetin-3-*O*-rhamnoside, owing the identification of these compound in leaves from *G. biloba* by Lin et al. (2008) and the latter one also by Yao et al. (2013).

Among the twenty-five compounds detected, compounds 1, 2, 4-6 and 12, associated to ginkgolides, were not quantified due their low UV absorption and possible interferences in the complex matrix of *G. biloba* extracts, as well as the unavailability of commercial gingkolide standards. Flavonoids were the main group present, being two kaempferol derivatives the majority compounds found (**Table 2**). Thus, kaempferol-3-*O*-dirhamnosylglucoside (compound **13**) was the most abundant compound in all the infusion preparations and in the methanol/water extract irradiated at 1 kGy, whereas kaempferol-3-*O*-rutinoside (compound **19**) was the most abundant one in the control and irradiated at 10 kGy methanol/water extracts. Protocatechuic acid was the only phenolic acid identified and the quantities present were in the same range as the major flavonoids.

This study intended to evaluate which irradiation dose would be the most efficient to improve the extractability of phenolic compounds in *G. biloba* samples (methanol/water extract and infusion oral solution). Infusions presented lower quantities than the

methanol/water extracts, due to the high temperatures applied to obtain these preparations that could destroy some thermal sensitive compounds, but also due to the lower extraction time. Both methanol/water and infusion preparation irradiated at a dose of 10 kGy gave the highest content in phenolic compounds. The sub-products formed during food irradiation depend on the food matrix and dose (Stewart, 2001). The degradation of some molecules during irradiation occurs by complex mechanisms. Although it is considered that some bonds can be broken resulting in smaller molecules (Stewart, 2001), the use of high irradiation doses might also lead to higher compound extractability. This could explain the higher values of phenolic compounds concentration observed for the doses of 10 kGy compared with those found in non-irradiated and 1 kGy irradiated samples. A small decrease in the phenolic compounds content was observed for the dose of 1 kGy in the methanol/water extract, when compared with non-irradiated samples. Low doses up to 1 kGy are used for preservation of fresh samples (Molins, 2001), which are more sensitive than dried food, indicating that at this doses there are no effect or only slight changes in food main characteristics.

To our knowledge, this is the first report that describes the phenolic composition in irradiated samples of *G. biloba* using two different doses. The dose of 10 kGy is enough to guarantee product disinfestation and microbial decontamination (Molins, 2001), contributing also for an increase in the phenolic compounds extractability, both for methanol/water and infusion preparations. The use of irradiation to improve bioactive properties was also suggested by other authors as a result of an increase in the levels of phenolic compounds in the extracts obtained from cooked and derived plant products (Zhu et al., 2010; Aouidi et al., 2011; Lee et al., 2013; Wanyo et al., 2014). Nevertheless, further research is needed to understand all the mechanisms involved in the irradiation processing effects in plant constituents.

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Table 1. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data and compound identification in *G. biloba* samples.

Compound	Rt (min)	λ_{max} (nm)	Molecular ion $[M-H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification
1	5.7	260,294,350	453	407(100),245(3),179(7),161(3),113(5)	Ginkgolide A derivative
2	6.1	358	449	403(16),269(4),205(8),179(5),113(4)	Unknown ginkgolide
3	6.2	262sh294	153	109(100)	Protocatechuic acid
4	6.8	252,356	325	163(100),119(87)	Bilobalide
5	7.1	274	407	245(100)	Ginkgolide A
6	9.3	282sh336	423	221(15),179(13),161(22),131(21),113(32)	Ginkgolide B
7	9.6	348	755	593(100),285(22)	Kaempferol-3-*O*-rhamnosylhexoside-7-*O*-glucoside
8	11.3	358	785	623(100),315(17)	Isorhamnetin-3-*O*-rhamnosylhexoside-7-*O*-glucoside
9	14.0	348	593	447(23),285(58)	Kaempferol-*O*-rhamnosyl-glucoside
10	15.0	354	755	301(100)	Quercetin 3-*O*-2'',6''-dirhamnosylglucoside
11	15.1	350	625	317(100)	Myricetin-3-*O*-rutinoside
12	16.5	318	439*[1]	411(18),383(93),365(12),322(26),304(7),277(7),259(8)	Ginkgolide C derivative
13	17.0	348	739	285(100)	Kaempferol-3-*O*-dirhamnosylglucoside
14	17.4	356	769	315(100)	Isorhamnetin-3-*O*-dirhamnosylglucoside
15	18.3	356	609	301(100)	Quercetin-3-*O*-rutinoside
16	19.1	360	639	331(100)	Patuletin-3-*O*-rutinoside
17	19.7	358	463	301(100)	Quercetin-3-*O*-glucoside
18	21.1	352	609	301(100)	Quercetin-3-*O*-glucosyl-(1,2)-rhamnoside
19	21.7	348	593	285(100)	Kaempferol-3-*O*-rutinoside
20	22.6	356	623	315(100)	Isorhamnetin-3-*O*-rutinoside

21	23.1	352	447	301(100)	Quercetin-3-*O*-rhamnoside
22	23.8	350	477	315(100)	Isorhamnetin-3-*O*-glucoside
23	25.1	348	593	285(100)	Kaempferol-3-*O*-glucosyl-(1,2)-rhamnoside
24	28.1	268,316	755	609(46),301(21)	Quercetin-3-*O*-*p*-coumaroyl-rhamnosylhexoside
25	30.8	266,316	739	593(51),285(21)	Kaempferol-3-*O*-*p*-coumaroyl-rhamnosylhexoside

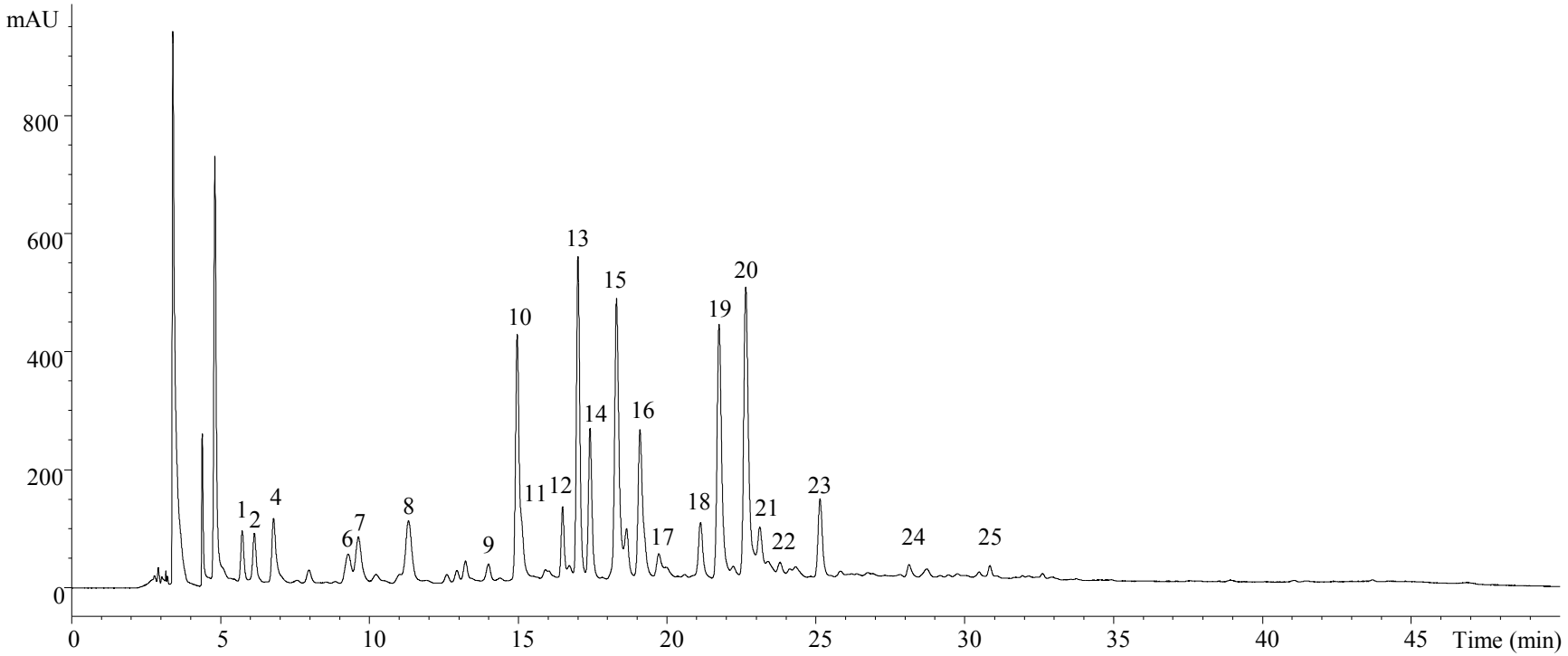
*[1] 879 $[2M-H]^-$

Table 2. Quantification of the phenolic compounds (mg/g of extract/lyophilized infusion) identified in methanol/water extracts and infusions of *G. biloba* non-irradiated and irradiated samples.

Compounds	Methanol/water extracts			Infusions		
	0 kGy	1 kGy	10 kGy	0 kGy	1 kGy	10 kGy
Ginkgolide A derivative	nq	nq	nq	nq	nq	nq
Unknown ginkgolide	nq	nq	nq	nq	nq	nq
Protocatechuic acid	1.39 ± 0.08	1.22 ± 0.04	4.49 ± 0.05	0.43 ± 0.04	0.38 ± 0.01	1.95 ± 0.05
Bilobalide	nq	nq	nq	nq	nq	nq
Ginkgolide A	nq	nq	nq	nq	nq	nq
Ginkgolide B	nq	nq	nq	nq	nq	nq
Kaempferol-3-*O*-rhamnosylhexoside-7-*O*-glucoside	0.34 ± 0.02	0.49 ± 0.01	1.16 ± 0.06	0.24 ± 0.02	0.25 ± 0.01	0.34 ± 0.05
Isorhamnetin-3-*O*-rhamnosylhexoside-7-*O*-glucoside	0.32 ± 0.04	0.45 ± 0.04	1.09 ± 0.14	0.22 ± 0.02	0.24 ± 0.02	0.35 ± 0.05
Kaempferol-*O*-rhamnosyl-glucoside	0.12 ± 0.02	0.16 ± 0.01	0.53 ± 0.11	0.03 ± 0.01	0.04 ± 0.00	0.11 ± 0.02
Quercetin 3-*O*-2’’,6’’-dirhamnosylglucoside	0.55 ± 0.01	0.66± 0.04	1.67 ± 0.07	0.17 ± 0.01	0.19 ± 0.01	0.56 ± 0.03
Myricetin-3-*O*-rutinoside	0.11 ± 0.01	0.07 ± 0.01	0.59 ± 0.08	nd	nd	0.12 ± 0.03
Ginkgolide C derivative	nq	nq	nq	nd	nq	nq
Kaempferol-3-*O*-dirhamnosylglucoside	1.26 ± 0.01	1.48 ± 0.02	3.57 ± 0.03	0.44 ± 0.02	0.40 ± 0.02	1.29 ± 0.01
Isorhamnetin-3-*O*-dirhamnosylglucoside	0.42 ± 0.01	0.51 ± 0.03	1.30 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.45 ± 0.01
Quercetin-3-*O*-rutinoside	0.84 ± 0.06	0.34 ± 0.02	2.74 ± 0.07	tr	0.07 ± 0.01	0.74 ± 0.03
Patuletin-3-*O*-rutinoside	0.52 ± 0.01	0.25 ± 0.01	1.89 ± 0.04	tr	0.04 ± 0.00	0.51 ± 0.03
Quercetin-3-*O*-glucoside	0.08 ± 0.01	tr	0.56 ± 0.05	nd	nd	0.05 ± 0.02
Quercetin-3-*O*-glucosyl-(1,2)-rhamnoside	0.20 ± 0.02	0.04 ± 0.01	0.78 ± 0.06	nd	tr	0.17 ± 0.01
Kaempferol-3-*O*-rutinoside	1.38 ± 0.04	0.57 ± 0.03	4.21 ± 0.02	0.02 ± 0.00	0.16 ± 0.02	1.15 ± 0.04
Isorhamnetin-3-*O*-rutinoside	1.06 ± 0.02	0.49 ± 0.03	3.05 ± 0.06	0.03 ± 0.00	0.16 ± 0.01	0.95 ± 0.01
Quercetin-3-*O*-rhamnoside	0.16 ± 0.01	0.09 ± 0.01	0.67 ± 0.05	nd	tr	0.17 ± 0.02
Isorhamnetin-3-*O*-glucoside	0.15 ± 0.04	0.12 ± 0.01	0.46 ± 0.05	nd	0.04 ± 0.00	0.16 ± 0.01
Kaempferol-3-*O*-glucosyl-(1,2)-rhamnoside	0.49 ± 0.02	0.15 ± 0.03	1.81 ± 0.15	0.02 ± 0.00	0.02 ± 0.00	0.38 ± 0.01
Quercetin-3-*O*-*p*-coumaroyl-rhamnosylhexoside	0.06 ± 0.01	0.04 ± 0.00	1.30 ± 0.08	tr	tr	0.10 ± 0.02

Kaempferol-3-*O*-*p*-coumaroyl-rhamnosylhexoside	0.11 ± 0.01	0.09 ± 0.01	1.83 ± 0.04	0.02 ± 0.00	0.01 ± 0.00	0.15 ± 0.01
Total phenolic acids	1.40 ± 0.08b	1.22 ± 0.04c	4.49 ± 0.05a	0.43 ± 0.04b	0.38 ± 0.01b	1.95 ± 0.05a
Total flavonoids	8.16 ± 0.04b	6.00 ± 0.18c	29.20 ± 0.74a	1.35 ± 0.01c	1.75 ± 0.06b	7.76 ± 0.16a
Total phenolic compounds	9.56 ± 0.06b	7.22 ± 0.22c	33.69 ± 0.69a	1.78 ± 0.06c	2.13 ± 0.04b	9.70 ± 0.11a

nd-not detected; nq- not quantified; tr-traces. In each row and for each extract (methanol/water extracts or infusions) different letters mean significant differences among total compounds ($p<0.05$).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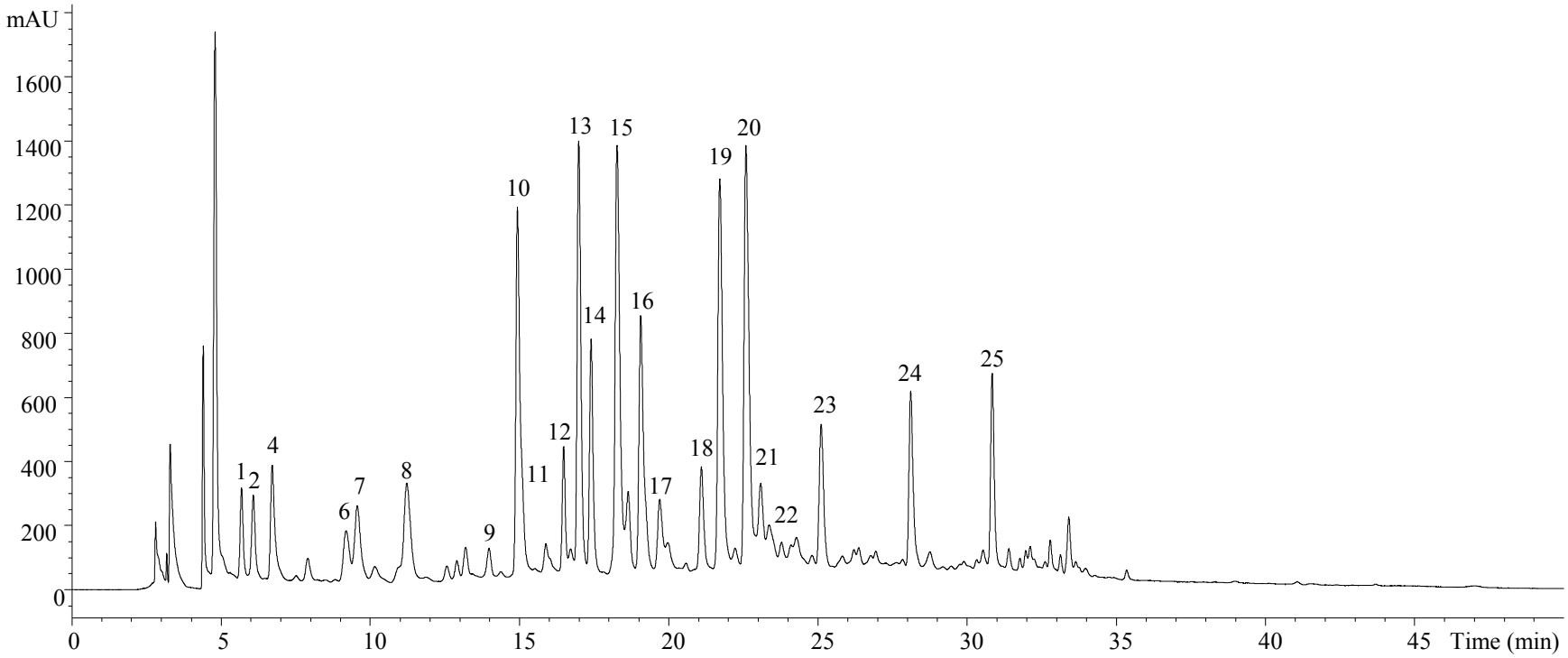
45
Time (min)

A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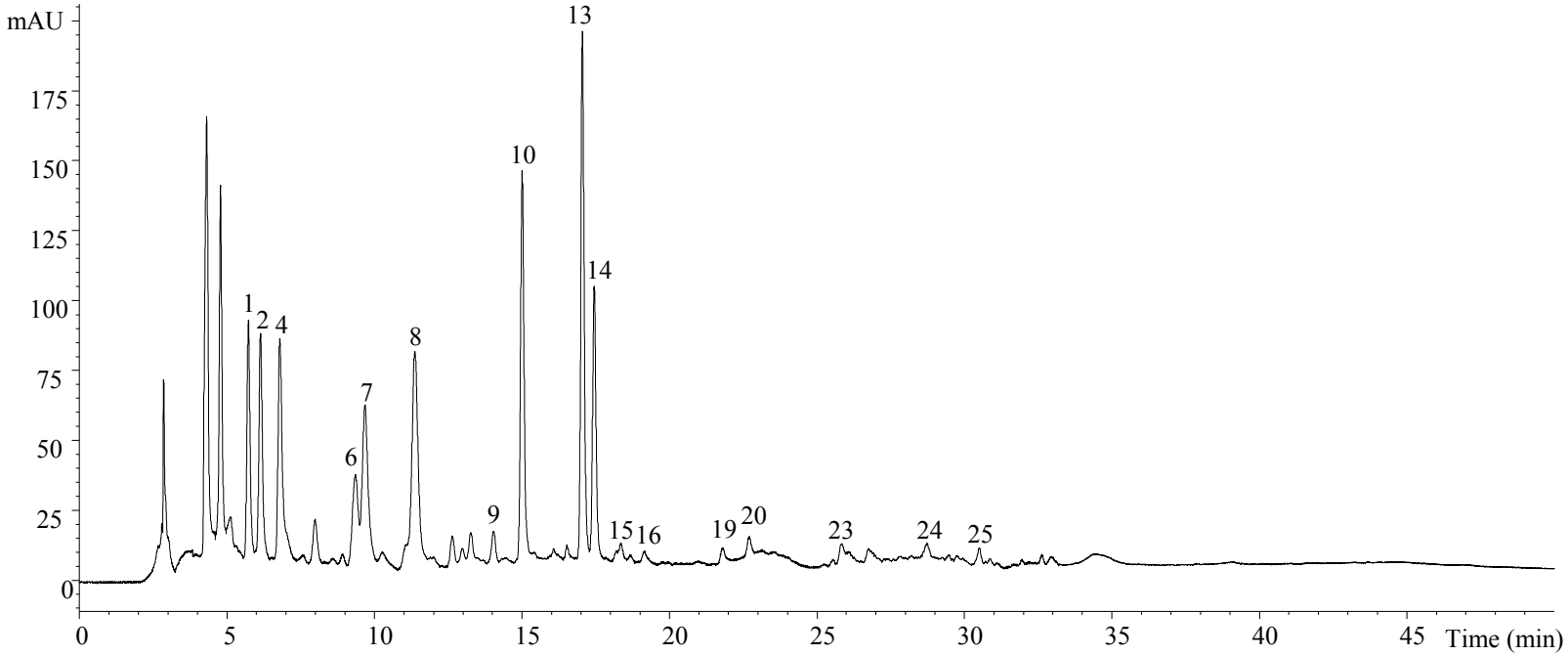
B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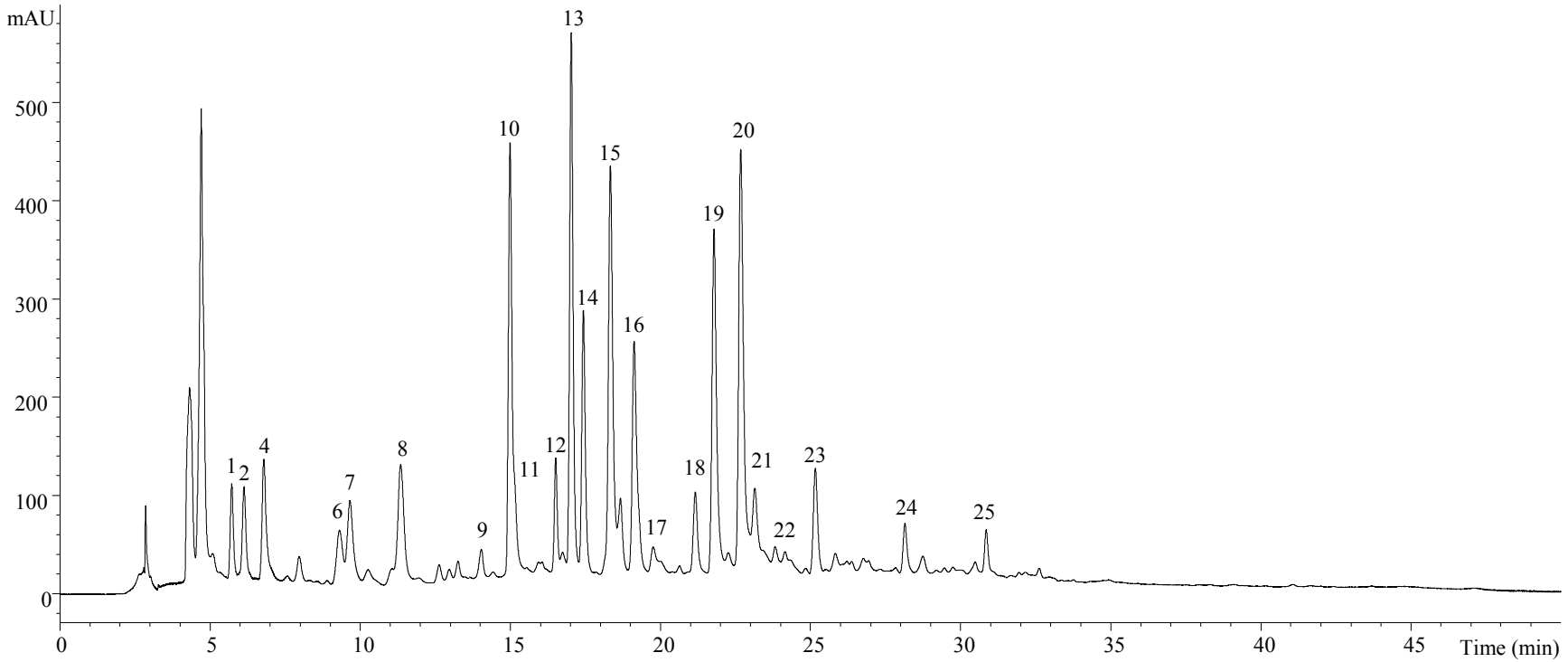
Time (min)

C



D

Figure 1. Chromatographic profile of non-irradiated methanol/water (A), irradiated at 10 kGy methanol/water (B), non-irradiated infusion (C) and irradiated at 10 kGy infusion (D) of *G. biloba* samples, recorded at 370 nm.