Protection of deoxyribose and DNA from degradation by using aqueous extracts of several wild plants

Maria S Gião,[1] M L González-Sanjosé,[2] Pilar Muñiz,[2] M D Rivero-Pérez,[2]
Monika Kosinska,[1] Manuela E Pintado[1] and F Xavier Malcata[1]*

[1]*Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal*
[2]*Department of Biotechnology and Food Science, Universidade de Burgos, Plaza Misael Bañuelos, E-09001 Burgos, Spain*

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Abstract

BACKGROUND: Aqueous extracts of 48 herbal plants were obtained via alternative extraction protocols, and were assayed for their capacity to protect deoxyribose and DNA itself from degradation (or, conversely, for their capacity to promote DNA degradation), using electrophoresis as analytical tool.

RESULTS: For a given (constant) volume of extract, deoxyribose protection ranged from 14.13 ± 1.35% (mean ± SD) inhibition by dwarf mallow powder infusion, up to 106.51 ± 15.93% inhibition by avocado powder infusion. DNA protection was tested at two extract concentrations, and was slightly greater at the higher concentration. Pro-oxidant effects were essentially absent.

CONCLUSION: The anti-oxidative roles of plants upon deoxyribose and DNA displayed by our experimental results were rather promising with regards to practical applications of those plants, viz. as ingredients in the formulation of nutraceutical beverages and/or foods.

Introduction

Oxidative stress has been implicated with pathogenesis of many diseases and health conditions, including (but not limited to) ageing, atheroschlerosis, cancer and respiratory diseases.[1] Such a form of stress occurs when excessive amounts of reactive oxygen species (ROS), viz. superoxide ($O_2^{\bullet -}$) and hydroxyl ($OH^{\bullet}$) radicals, over-run the antioxidant defenses of the host, thus producing (often irreversibly) cellular damage.

Note that ROS are normally generated, by all aerobic organisms, as (unwanted) by-products of their regular oxygen metabolism, and those organisms accordingly synthesise antioxidants, aimed at eventually inhibiting the damages brought about by ROS in their living tissues. As long as adequate amounts of antioxidants are produced, an oxidant–antioxidant balance can be maintained, so sufficient protection will be assured. However, if antioxidant protection is hampered due to disease, poor diet, risky lifestyle or other unfavourable environmental factors, net oxidative damage will take place.[2]

Antioxidants extracted from natural sources have been thoroughly studied in recent decades, owing to their beneficial effects upon human health. Those compounds are frequently found in plants, particularly in herbs.[3–10] However, under specific conditions, antioxidants can behave as free radicals, and hence lead to oxidation, thus exhibiting a pro-oxidant effect, which is often concentration dependent. Therefore, both favourable and unfavourable features of natural antioxidants should be taken into account, before an informed decision can be taken regarding their applications and usefulness.

By definition, an antioxidant is a compound which, when present at concentrations much lower than those of oxidisable substrate(s), delays to a significant extent, or even inhibits, oxidation of the substrate(s). Mechanisms by which antioxidants act include decrease of local concentration, or even depletion of O_2; reduction of catalytic metal ions; removal of such ROS as $O_2^{\bullet -}$ and H_2O_2; scavenging of initiating radicals, viz. $OH^{\bullet}$, $RO^{\bullet}$ and $RO_2^{\bullet -}$; disruption of chain reactions that have already been initiated; quenching of singlet oxygen; promotion of endogenous antioxidant defenses, via up-regulating the expression of genes encoding antioxidant enzymes; repair of oxidative damage brought about by radicals; enhancing the elimination of damaged molecules; and by-passing

* Correspondence to: F Xavier Malcata, Escola Superior de Biotecnologia, Rua Dr. António Bernardino de Almeida, P-4200-072 Porto, Portugal
E-mail: fxmalcata@esb.ucp.pt
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the repair of excessively damaged molecules, thus minimising the incidence of mutations.[1,2,11–13] Hence, the most effective antioxidants are those that are able to perform more than one of the aforementioned roles, without concomitantly generating toxic, or otherwise reactive, end-products.

Total antioxidant capacity can be ascertained by methods based on 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation ($ABTS^{\bullet +}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) or oxygen radical absorbance capacity (ORAC) as substrates,[11–14] among others; these primary, *in vitro* approaches have then to be complemented with *in vivo* studies, when an assessment of bioavailability and bioactivity is sought. In the case of protection of DNA from degradation, *in vitro* studies include tests based on such simple molecules as deoxyribose (the backbone sugar in DNA),[15–17] as well as lipid peroxidation; *in vivo* studies may include assessment of antitumour, antiplatelet, antiallergic, antischaemic and anti-inflammatory activities,[18] which will typically resort to animal or human models, in attempts to determine the antioxidant or pro-oxidant potential under live physiological conditions.[19]

When assaying for antioxidant capacity, hydroxyl radicals are typically generated within a mixture of ascorbic acid, H_2O_2 and Fe^{3+}-ethylenediaminetetracetic acid (EDTA); those radicals that are not scavenged by other components of the reaction mixture will eventually attack deoxyribose, thus degrading it into a series of fragments. Some of the fragments (or even all of them) react upon heating with thiobarbituric acid (TBA), at low pH, thus yielding a pink chromogen: this TBA adduct possesses a three-carbon dialdehyde, malondialdehyde (MDA). If an $OH^\bullet$ scavenger is meanwhile added to the reaction mixture, it will compete with deoxyribose for $OH^\bullet$ radicals, and consequently inhibit deoxyribose degradation.[20–22] Despite concerns regarding the specificity and validity of the TBA assay, viz. possible interference with haemoglobin or biliverdin present in the sample, potential thermal degradation due to heating during the assay, presence of iron in the assay reagents, rapid metabolism of MDA, and low representativeness of MDA among lipid peroxides (less than 1%),[2] the assay is still chosen by several researchers and is thus useful for comparative purposes. Furthermore, $OH^\bullet$ radicals can also enhance DNA damage, via attack on its phosphate bonds; this type of degradation results in smaller fragments, which can be separated by agarose electrophoresis.[21,22]

Consequently, the aim of this work was to assess the capacity of a number of native plants to prevent oxidative degradation of deoxyribose and DNA, and also their possible pro-oxidant effect upon DNA. All previous reports (when available) that refer to the plants selected for our study have not conveyed any data on the protection of those important moieties with roles of transmission of genetic information. This screening is crucial in attempts to ascertain the antioxidant potential of these plants, for eventual use in food and cosmetic formulations.

Materials and Methods

Sample preparation

Forty-eight distinctive plants, which essentially cover the whole range of plants commonly used in Portugal for traditional medicine, were provided by ERVITAL (Castro Daire, Portugal). All were produced via organic farming, and were picked out at random. The plants were supplied in dried leaf form, following harvesting at the developmental stage known to maximise their putative therapeutic activity (which is the form presented to consumers); a sufficiently large portion was crushed (using a coffee mill) in order to obtain the corresponding powder. In the case of the preparations based on plain infusion, 110 mL of boiling distilled water was added to 1 g of leaves and/or powder and, after 5 min, the mixture was filtered through a 0.45 μm filter. In the case of a preparation that required boiling, 110 mL of distilled water was added to 1 g of leaves, the mixture was boiled for 5 min and then filtered through a 0.45 μm filter. For assays encompassing deoxyribose, plain extracts (obtained as described above) were used. For assays encompassing DNA, each plain extract was first concentrated 8-fold, then 40 mL of the concentrated extract was frozen and lyophilised. It was reconstituted with 5 mL of ultra-pure water prior to the analysis.

Chemicals and reagents

Ascorbic acid, EDTA, 2-deoxy-D-ribose (deoxyribose), calf thymus DNA, agarose and bromophenol blue where purchased from Sigma–Aldrich (Steinheim, Germany); TBA was obtained from Merck (Damstadt, Germany); NaH_2PO_4, Na_2HPO_4, Fe_2Cl_3, 33% (v/v) H_2O_2, TCA, glycerol and $CuSO_4$ were supplied by Panreac (Barcelona, Spain); and ethidium bromide was from Amresco (Solon, OH, USA).

Assay for effects on deoxyribose

Deoxyribose protection was quantified by using the methods described by Guimarães *et al.*[16] and Halliwell *et al.*[17] Aliquots (100 μL) of $0.5\,g\,L^{-1}$ standard solutions of ascorbic, ferulic and chlorogenic acids were studied in the same way as the actual samples. A 100 μL sample of the extract of interest was added to 10 μL of $100\,mmol\,L^{-1}$ deoxyribose, and then incubated for 1 h at 37 °C in the presence of 10 μL of $10\,mmol\,L^{-1}\,Fe^{3+}$, 10 μL of $1\,mmol\,L^{-1}\,H_2O_2$ 33% and 10 μL of $10\,mmol\,L^{-1}$ EDTA, in a $24\,mmol\,L^{-1}$ sodium phosphate buffer (pH 7.4) containing $15\,mmol\,L^{-1}$ NaCl in order to generate hydroxyl radicals. As mentioned above, radicals break deoxyribose into fragments which, in the presence of 1 mL of 1% TBA in $0.05\,mol\,L^{-1}$ NaOH, under acid conditions (1.5 mL of 28% TCA) and at high temperature (100 °C for 15 min), give rise to a

chromophore (malonaldehyde), and this species was quantified by absorbance at 532 nm, using a Heλios α spectrophotometer (Unicam, Cambridge, UK). When antioxidants are present, they compete for the hydroxyl radicals, thus decreasing the extent of fragmentation of deoxyribose. All measurements were made against adequate blanks, as originally described by Halliwell *et al*.[17] Triplicate samples were used, and for each one, analyses were run in quadruplicate.

Assay for effects on DNA

DNA protection was quantified using the method described by Rivero *et al*. and Guimarães *et al*.[15,16] A 200- or 400-μL aliquot of the sample was initially prepared and processed as detailed below, as well as 150 μL of 0.5 g L^{-1} standard solutions of ascorbic, ferulic and chlorogenic acids. This method includes assays of both controls and samples, and assesses both the anti- and pro-oxidant capacities of the latter. Different steps were considered for this purpose: (1) positive control, using 800 μL of 100 mmol L^{-1} sodium phosphate buffer (pH 7.4) and 200 μL of 1 mg mL^{-1} DNA; (2) negative control, using 690 μL of 100 mmol L^{-1} sodium phosphate buffer (pH 7.4), 200 μL of 1 mg mL^{-1} DNA, 100 μL of 1 mmol L^{-1} ascorbic acid and 10 μL of 100 mmol L^{-1} Cu^{2+}; (3) antioxidant effect of samples, using 490 or 290 μL of 100 mmol L^{-1} sodium phosphate buffer (pH 7.4), 200 μL of 1 mg mL^{-1} DNA, 200 or 400 μL of sample, 100 μL of 1 mmol L^{-1} ascorbic acid and 10 μL of 100 mmol L^{-1} Cu^{2+}; and (4) pro-oxidant effect of samples, using 600 or 800 μL of 100 mmol L^{-1} sodium phosphate buffer (pH 7.4), 200 μL of 1 mg mL^{-1} DNA, and 200 or 400 μL of sample. The final volume was, in all cases, adjusted to 1 mL with buffer solution, and the mixtures were incubated for 1 h at 37 °C. Following incubation, 50 μL aliquots were mixed with 10 μL of buffer: 20% (w/v) glycerol containing 0.1% (w/v) bromophenol blue, and placed on top of wells of 0.7% (w/v) agarose gel. This gel had been prepared with 100 mmol L^{-1} sodium phosphate buffer (pH 7.4), containing 5 mmol L^{-1} EDTA. After running, the gel was submerged in a 10 mg L^{-1} solution of ethidium bromide. Electrophoresis was run using a Bio-Rad (Richmond, CA, USA) power supply model 1000/500, at 400 V and 400 mA. DNA bands were finally digitalised using Gel Doc (from Bio-Rad).

Statistical analyses

Non-parametric tests were applied to each set of experimental data pertaining to deoxyribose, to show whether the type of plant, its degree of division and the form of extraction were statistically significant parameters. To study the correlation between the two methods used, a non-linear canonic correlation (OVERALS) was applied, with deoxyribose data being categorised in three groups (<40%, 40–60% and >60% inhibition), and DNA results classified as positive or negative, depending on whether they exhibited antioxidant activity or not. All analyses were carried out using SPSS v. 15.0.0 (Chicago, IL, USA).

Results and discussion

All plants tested were previously described in terms of total antioxidant capacity and phenolic content,[3] but not with regard to the specific consequences of bulk activity on deoxyribose and DNA protection. Furthermore, the overall antioxidant capacity was statistically correlated by those authors to the protective principles observed, so it was not repeated here.

The data generated pertaining to deoxyribose protection are given in Table 1. Major differences can easily be seen: the protection ranged from $106.51 \pm 15.93\%$ inhibition for avocado (*Persea americana*, Lauraceae) down to $14.13 \pm 1.35\%$ inhibition for dwarf mallow (*Malva silvestris,* Malvacea). Both samples were obtained by powder infusion and each result is given as the mean ± SD.

With regard to pure compounds, ascorbic acid presented a protection of $39.84 \pm 20.92\%$, ferulic acid of $50.65 \pm 6.09\%$ and chlorogenic acid of $44.57 \pm 9.00\%$. The great deviation observed for ascorbic acid relative to the mean value is possibly due to the antioxidant activity of this compound (which is not very stable) when used in this method. In general, the highest deoxyribose protection was provided by avocado, followed by red centaury (*Erythraea centaurium*, Gentianaceae), white Spanish broom (*Cytisus multiflorus*, Fabaceae), sweet amber (*Hypericum androsaemum*, Clusiaceae), lovage (*Levisticum officinale*, Apiaceae), European pennyroyal (*Mentha pulegium*, Lamiacea), savory (*Satureja montana*, Lamiacea), linden tree (*Tilia cordata*, Tiliaceae), thyme (*Thymus vulgaris*, Lamiacea) and lemon thyme (*Thymus citriodorus*, Lamiacea), listed by decreasing degree of protection.

Avocado yielded unique and particularly interesting results, especially when considering that little information had to date been made available concerning that plant. Besides a strong protection of deoxyribose, this plant also exhibits a high antioxidant capacity (1.4280 ± 0.1261 g L^{-1} equivalent of ascorbic acid) and a high content of total phenolic compounds (0.5541 ± 0.0289 g L^{-1} equivalent of gallic acid).[3]

From a statistical point of view, deoxyribose protection was not characterised by a high discriminating power among plants, since only about 38% of the plants were statistically different between them, at a level of significance of 0.05. (Recall that non-parametric tests were applied to the experimental data, because they were not normally distributed, as such.) Furthermore, boiling was statistically different from infusion of leaves, as well as powder relative to plain leaf infusion; however, powder infusion was not statistically different from boiling of leaves, at the above level of significance.

In what concerns anti- or pro-oxidant effects upon DNA, data pertaining to standards are shown in

Table 1. Protection of deoxyribose (quantitative) from oxidation (average ± standard deviation), and anti- and pro-oxidant effects on DNA (qualitative) of several plant extracts

		Infusion of powder					Infusion of leaves					Boiling of leaves				
		Deoxyribose	DNA (200 µL)		DNA (400 µL)		Deoxyribose	DNA (200 µL)		DNA (400 µL)		Deoxyribose	DNA (200 µL)		DNA (400 µL)	
Scientific name (family)	English common name	(% inhibition)	A	P	A	P	(% inhibition)	A	P	A	P	(% inhibition)	A	P	A	P
Achillea millefolium (Asteraceae)	Yarrow	49.37 ± 6.35	+	−	−	−	52.64 ± 9.43	−	−	−	−	55.49 ± 9.41	−	−	+	−
Agrimonia eupatoria (Rosaceae)	Agrimony	66.16 ± 8.33	−	−	−	−	32.99 ± 7.31	−	−	−	−	47.02 ± 4.32	−	−	−	−
Artemisia dracunculus (Asteraceae)	Tarragon	48.02 ± 9.29	+	−	−	−	53.81 ± 11.12	−	−	−	−	50.24 ± 7.80	−	−	−	−
Betula pendula (Betulaceae)	Birch	54.42 ± 4.16	−	−	−	−	42.71 ± 12.15	−	−	−	−	45.21 ± 11.89	+	−	+	−
Calluna vulgaris (Ericaceae)	Heath	57.86 ± 16.87	−	−	+	−	30.75 ± 15.35	−	−	−	−	51.95 ± 7.88	−	−	−	+
Chamaemelum nobile (Asteraceae)	Wild camomile	66.78 ± 7.05	+	−	+	−	59.16 ± 4.35	−	−	−	−	46.56 ± 6.82	−	−	−	−
Chamaespartium tridentatum (Leguminosae)	Winged broom	43.10 ± 7.19	+	−	+	−	49.42 ± 10.90	−	−	−	−	59.83 ± 6.04	−	−	−	−
Chamomilla sp. (Asteraceae)	Chamomile	44.38 ± 10.24	+	−	+	−	50.66 ± 6.33	−	−	−	−	48.93 ± 6.91	+	−	+	−
Coriandrum sativum (Apiaceae)	Coriander	35.51 ± 9.65	−	−	−	−	23.00 ± 5.41	+	−	−	−	51.46 ± 6.91	−	−	−	−
Cymbopogon citratus (Poaceae)	Lemon grass	59.08 ± 18.38	+	−	+	−	33.83 ± 5.83	−	−	−	−	41.56 ± 3.53	+	−	−	−
Cynara cardunculus (Asteraceae)	Artichoke	51.53 ± 4.59	+	−	+	−	15.74 ± 2.92	+	−	+	−	54.21 ± 6.52	+	−	+	−
Cytisus multiflorus (Fabaceae)	White Spanish broom	70.69 ± 9.45	+	−	+	−	54.84 ± 10.86	+	−	+	−	46.09 ± 7.20	+	−	+	+
Echinacea purpurea (Asteraceae)	Echinacea	53.74 ± 2.44	+	−	+	−	20.04 ± 1.67	−	−	−	−	30.45 ± 7.13	−	−	−	−
Equisetum arvense (Equisetaceae)	Horsetail	63.21 ± 10.43	+	−	−	−	32.90 ± 13.17	−	−	−	−	67.66 ± 15.04	−	−	−	−
Erythraea centaurium (Gentianaceae)	Red centaury	73.27 ± 5.97	−	−	−	−	62.85 ± 10.87	−	−	−	−	66.95 ± 5.80	−	−	+	−
Eucalyptus globulus (Myrtaceae)	Eucalyptus	50.42 ± 8.58	−	−	−	−	29.05 ± 4.30	−	−	−	−	34.95 ± 9.77	−	−	−	−
Filipendula ulmaria (Rosaceae)	Queen-of-the meadow	21.16 ± 11.73	−	−	−	−	44.57 ± 35.92	−	−	+	−	42.83 ± 3.87	−	−	+	+
Foeniculum vulgare (Apiaceae)[l]	Fennel	51.03 ± 6.76	−	−	−	−	31.40 ± 5.28	−	−	−	−	51.24 ± 4.27	−	−	+	−
Fraxinus (Oleaceae)	Ash	43.52 ± 8.04	−	++	−	−	42.52 ± 2.39	−	−	−	−	59.00 ± 7.33	−	−	−	−
Hypericum androsaemum (Clusiaceae)	Sweet amber	33.68 ± 2.75	+	−	−	−	57.13 ± 9.97	−	−	−	−	76.06 ± 9.61	−	−	−	−
Hypericum perforatum (Clusiaceae)	St John's wort	56.73 ± 16.98	−	−	−	−	102.51 ± 2.91	−	−	−	−	22.73 ± 3.52	−	−	−	−
Hyssopus officinalis (Lamiaceae)	Hyssop	59.45 ± 3.81	−	−	−	−	31.06 ± 6.08	−	−	−	−	39.56 ± 4.11	+	−	+	+
Juglans regia (Juglandaceae)	Walnut tree	45.90 ± 13.44	−	−	−	−	53.24 ± 4.17	−	−	−	−	51.87 ± 8.51	−	−	−	−

Laurus nobilis (Lauraceae)	Laurel	41.60 ± 6.82	−	−	−	−	33.23 ± 5.22	−	−	−	−	78.12 ± 6.07	−	−	−	−
Lavandula officinalis (Lamiaceae)	Lavender	33.71 ± 9.48	−	−	−	−	44.22 ± 8.94	−	−	−	−	67.01 ± 4.44	−	−	−	−
Lavandula stoechas (Lamiaceae)	French lavender	61.54 ± 11.57	−	−	−	−	53.97 ± 9.66	−	−	−	−	62.54 ± 5.98	−	−	−	−
Levisticum officinale (Apiaceae)	Lovage	70.28 ± 16.72	+	−	+	−	40.53 ± 9.55	−	+	−	−	55.71 ± 6.03	+	−	+	−
Lippia citriodora (Verbenaceae)	Lemon verbena	31.54 ± 6.92	−	−	−	−	30.09 ± 6.77	−	−	−	−	40.62 ± 10.89	−	−	+	−
Malva silvestris (Malvaceae)	Dwarf mallow	14.13 ± 1.35	+	−	+	−	47.67 ± 6.89	−	−	−	−	39.38 ± 7.35	+	−	+	−
Matricaria chamomilla (Asteraceae)	Chamomile	60.87 ± 11.64	+	−	+	−	58.15 ± 14.05	−	−	+	−	73.23 ± 6.95	+	−	+	−
Mentha piperita (Lamiaceae)	Peppermint	48.16 ± 9.97	−	+	−	−	57.59 ± 9.92	−	−	+	−	52.00 ± 5.55	−	−	−	−
Mentha pulegium (Lamiacea)	European pennyroyal	70.83 ± 19.19	+	−	+	−	43.67 ± 13.31	−	−	+	−	49.65 ± 7.04	−	−	+	−
Mentha spicata (Lamiaceae)	Spearmint	44.53 ± 10.43	−	−	++	−	41.07 ± 3.96	−	−	−	−	67.29 ± 4.91	−	−	−	−
Myrtus communis (Myrtaceae)	Myrtle	50.08 ± 12.16	−	−	−	−	28.28 ± 3.54	−	−	−	−	45.36 ± 5.47	−	−	−	−
Ocimum basilicum (Lamiaceae)	Sweet basil	32.64 ± 3.08	−	−	−	−	42.68 ± 9.28	−	−	−	−	52.38 ± 2.19	−	−	−	−
Olea europaea (Oleaceae)	Olive leaf	49.55 ± 9.21	−	−	−	−	28.34 ± 5.78	+	−	+	−	29.21 ± 5.00	−	−	+	−
Persea americana (Lauraceae)	Avocado	106.51 ± 15.93	−	−	−	−	56.43 ± 28.14	−	−	−	−	40.73 ± 10.75	−	−	−	−
Rosmarinus officinalis (Lamiaceae)	Rosemary	15.74 ± 2.98	−	−	−	−	21.39 ± 7.71	−	−	−	−	31.34 ± 5.15	−	−	−	−
Rubus idaeus (Rosaceae)	Raspberry	55.73 ± 8.55	+	−	+	−	50.13 ± 9.93	−	−	−	−	48.96 ± 12.85	−	−	−	−
Salvia sp. (Lamiaceae)	Sage	34.22 ± 8.93	−	−	−	−	24.54 ± 1.48	−	−	−	−	45.29 ± 2.25	−	−	−	−
Sambucus nigra (Caprifoliaceae)	Black elder flowers	35.38 ± 9.34	+	−	+	−	64.02 ± 12.53	+	−	+	−	49.82 ± 3.21	+	+	+	−
Satureja montana (Lamiaceae)	Savory	53.82 ± 5.81	−	−	−	−	51.88 ± 8.32	−	−	−	−	72.05 ± 3.46	−	−	−	−
Thymus vulgaris (Lamiaceae)	Thyme	72.29 ± 8.43	+	−	+	−	41.99 ± 14.27	−	−	−	−	51.38 ± 4.53	−	−	−	−
Thymus mastichina (Lamiacea)	Spanish wood marjoram	28.59 ± 5.73	−	−	−	−	45.45 ± 9.34	+	−	−	−	44.47 ± 2.99	−	−	+	+
Thymus citriodorus (Lamiacea)	Lemon thyme	72.60 ± 7.34	−	−	+	−	65.87 ± 10.87	−	−	+	−	71.23 ± 13.52	+	−	+	−
Tilia cordata (Tiliaceae)	Linden tree	90.36 ± 14.47	−	−	+	−	30.13 ± 14.45	−	−	−	−	61.99 ± 6.35	−	−	+	+
Urtica dioica (Urticaceae)	Nettles	44.70 ± 11.80	+	−	++	−	40.90 ± 14.65	+	−	+	−	54.76 ± 6.08	+	−	+	−
Zea mays (Poaceae)	Maize stigmae	56.42 ± 7.52	+	−	+	+	40.01 ± 9.61	+	−	−	−	30.15 ± 7.57	+	−	−	−

Note: $^{-}$ no effect, $^{+}$ weak effect, $^{++}$ strong effect.
A, antioxidant; P, pro-oxidant.

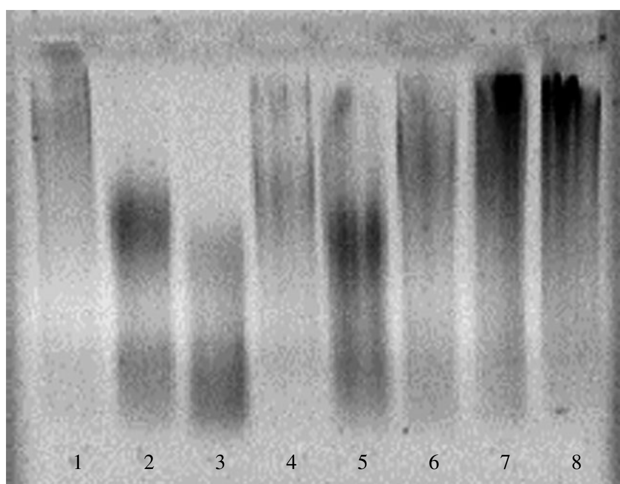

Figure 1. Effect of each standard, at various concentrations, upon DNA oxidative damage (induced by Cu(II)-ascorbic acid) and protection, assessed by agarose gel electrophoresis. DNA alone (lane 1); DNA + Cu(II)-ascorbic acid (lane 2); DNA + Cu(II)-ascorbic acid + 150 µL of 0.5 g L^{-1} ascorbic acid (lane 3), or 150 µL of 0.5 g L^{-1} ferulic acid (lane 4) or 150 µL of 0.5 g L^{-1} chlorogenic acid (lane 5); DNA + 150 µL of 0.5 g L^{-1} ascorbic acid (lane 6), or 150 µL of 0.5 g L^{-1} ferulic acid (lane 7) or 150 µL of 0.5 g L^{-1} chlorogenic acid (lane 8).

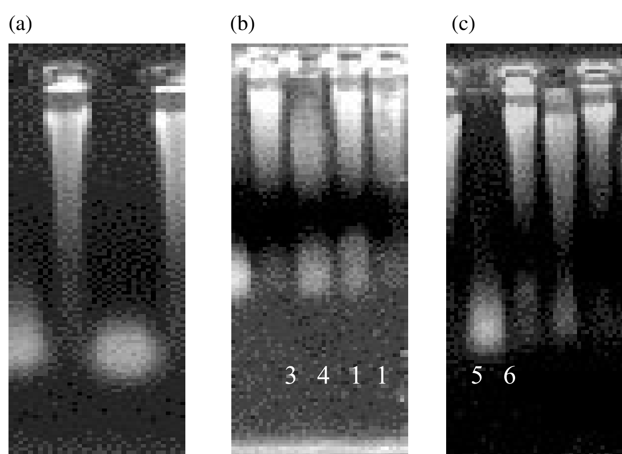

Figure 2. Effect of selected samples, labelled as no effect (–), weak effect (+) and strong effect (++) in Table 1, upon DNA oxidative damage (induced by Cu(II)-ascorbic acid) and protection, assessed by agarose gel electrophoresis. (a) DNA alone (lane 1) and DNA + Cu(II)-ascorbic acid (++; lane 2); (b) DNA + Cu(II)-ascorbic acid + 400 µL spearmint powder infusion (++; lane 3) and DNA + 400 µL spearmint powder infusion (+; lane 4); (c) DNA + Cu(II)-ascorbic acid + 200 µL avocado powder infusion (–; lane 5); and DNA + 200 µL avocado powder infusion (–; lane 6).

Fig. 1, whereas those pertaining to actual samples are presented in Table 1; selected data encompassing those samples featuring the poorest and strongest anti- and pro-oxidant effects are also shown in Fig. 2. From Fig. 1 it can be concluded that neither ascorbic, ferulic or chlorogenic acid display a pro-oxidant effect, at least at the levels tested. Ferulic acid yielded the greatest protection, followed by chlorogenic acid; ascorbic acid was unable to protect against DNA fragmentation, which was expected owing to its catalytic behaviour as an oxidant when in the presence of Cu.[23] Figure 2 illustrates the strong antioxidant (lane 3) and weak pro-oxidant (lane 4) effects of 400 µL powder infusion of spearmint, but no antioxidant (lane 5) or pro-oxidant (lane 6) effects of 200 µL of avocado powder infusion.

The strongest antioxidant effect upon DNA was again associated with powder infusion, whereas the lowest was observed for leaf infusion. As already pointed out,[3] an increase of specific area seems to significantly influence the degree of antioxidant extraction: this was somewhat expected, because a higher specific area will be available for mass transfer. However, the reverse holds in the case of sweet amber, dwarf mallow and sweet basil; in these cases, the effect of temperature appears to be more relevant toward liquid extraction.

Increasing the extract volume from 200 to 400 µL did not significantly affect the extent of protection of DNA, and no pro-oxidant effect was observed, except in the cases of ash (*Fraxinus*, Oleaceae) and hyssop (*Hyssopus officinalis*, Lamiaceae). The strongest protection was attained with 400 µL of powder infusion of spearmint (*Mentha spicata*, Lamiaceae) and nettles (*Urtica dioica*, Urticaceae). Catechin, epicatechin and gallic acid, at 0.5 mmol L^{-1}, were reported[8] to inhibit cleavage of DNA strands, induced by 0.5 mmol L^{-1} peroxynitrite, by no less than 90%; these compounds also reduce the frequency of DNA strand breaks, when in the presence of nitroxyl anion. Furthermore, such polyphenols as epicatechin, epigalocatechin gallate, epigalocatechin and epicatechin gallate from green tea yielded a protective effect *in vitro* upon oxidative damage of DNA, when induced by hydroxyl radicals.[24]

A non-linear canonic correlation, based on the generic principles of principal component analysis, was developed in two dimensions (with correlation coefficients for dimensions 1 and 2 of 0.478 and 0.332, respectively), with a fit value of 1.40 (70% of quality); these represent two groups of mutually correlated variables that are essentially independent (or orthogonal) from each other. Inspection of Fig. 3(a) indicates that dimension 1 discriminates between positive and negative results for antioxidant capacity (the weighted combination of the variables explains 74% of all variance amongst data), whereas inspection of Fig. 3(b) indicates that negative and positive results pertaining to DNA protection are closely associated to the intermediate values (40–60% inhibition) of deoxyribose protection, except for leaf infusion. Therefore, the two analytical methods employed are closely correlated with each other.

Conclusions

The technological process of aqueous extraction of the various plants affects the extent of recovery of compounds bearing antioxidant features: infusion of plant leaves in powder form is, in general, the most effective method, likely because the

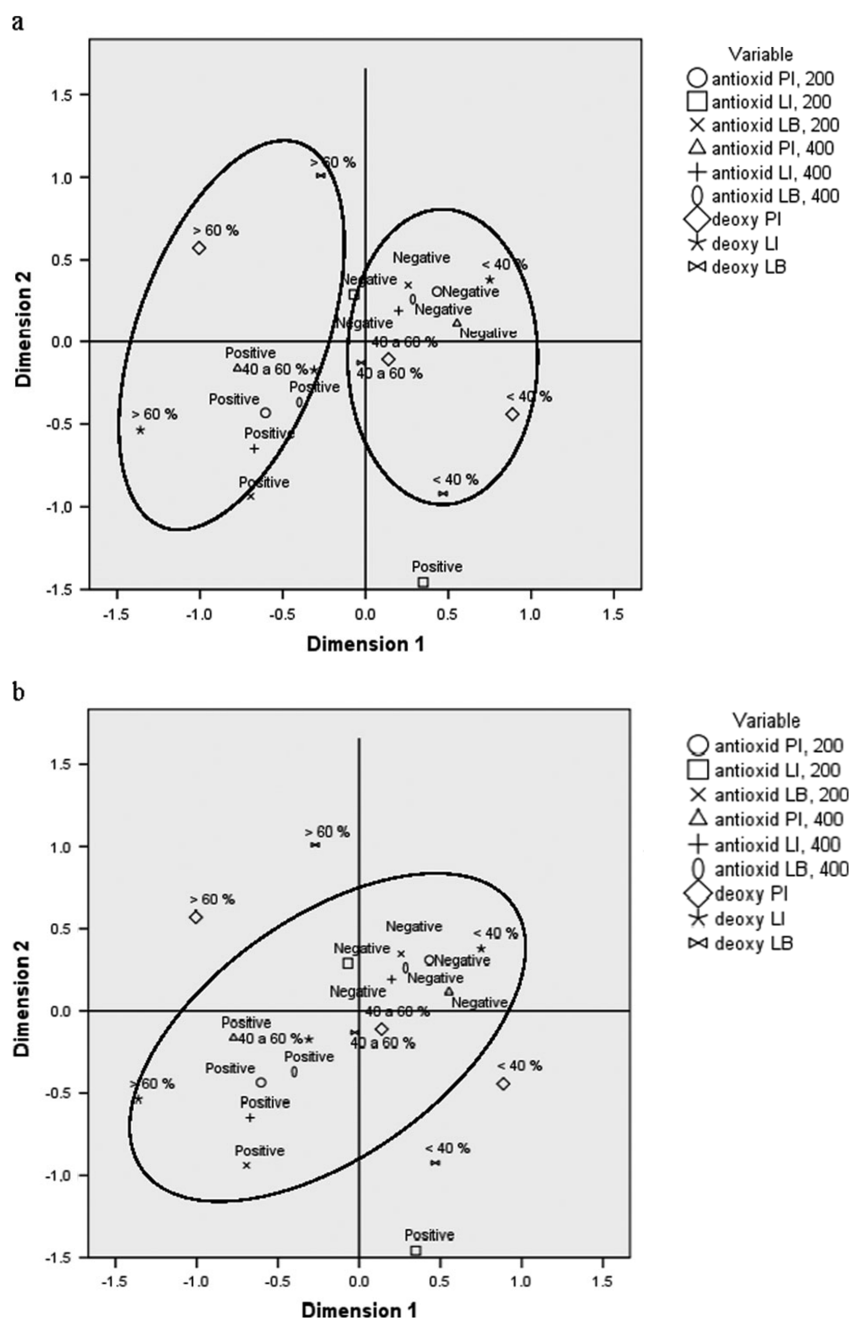

Figure 3. Canonical plots pertaining to deoxyribose and DNA analytical methods. Antioxid PI, 200 or 400: antioxidant effect observed in DNA test for powder infusion, using 200 or 400 μL, respectively, of sample; antioxid LI, 200 or 400: antioxidant effect observed in DNA test for leaf infusion, using 200 or 400 μL, respectively, of sample; antioxid LB, 200 or 400: antioxidant effect observed in DNA test for leaf boiling using 200 or 400 μL, respectively, of sample; deoxy PI: antioxidant effect observed in deoxyribose test for powder infusion; deoxy LI: antioxidant effect observed in deoxyribose test for leaf infusion; and deoxy LB: antioxidant effect observed in deoxyribose test for leaf boiling.

specific area of the feedstock plays a more relevant role in extraction than temperature of the solvent.

The highest degree of protection of deoxyribose occurs for powder infusion of avocado (106.51% inhibition) and of linden tree (90.36%); leaf boiling of sweet amber (76.06%); powder infusion of red centaury (73.27%), lemon thyme (72.60%) and thyme (72.29%); leaf boiling of savory (72.05%) and lemon thyme (71.23%); and powder infusion of European pennyroyal (70.83%), white Spanish broom (70.69%) and lovage (70.28%), listed by decreasing order. On the other hand, the highest degree of protection of DNA is observed for powder infusion of spearmint and nettles. The deoxyribose and DNA protection methods are strongly correlated with each other. Our results display a high potential of the Portuguese plants tested for functional food (or cosmetic) formulations, should a protective effect on biological samples be sought.

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