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Infusions of Portuguese medicinal plants: Dependence of final antioxidant capacity and phenol content on extraction features

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Abstract

BACKGROUND: Aqueous extracts of most medicinal plants traditionally employed in Portugal (at the ratio of 1 g plant: 110 mL water) have been assayed for total antioxidant capacity and phenol content, in order to elucidate their claimed medicinal features.

RESULTS: The antioxidant activity was assessed by the $ABTS^{\bullet+}$ method; the ascorbic acid equivalent values ranged from 1.4280 ± 0.1261 g L^{-1} for avocado (*Persea americana* (Lauraceae)) obtained by infusion of powder, down to 0.0027 ± 0.0012 g L^{-1} for olive (*Olea europaea* (Oleaceae)) obtained by infusion of leaves. Total phenol content was determined by the Folin–Ciocalteu procedure; the gallic acid equivalent values ranged from 0.5541 ± 0.0289 g L^{-1} for avocado obtained by infusion of powder, down to 0.0053 ± 0.0014 g L^{-1} for olive obtained by boiling leaves. A good correlation between total antioxidant capacity and total phenol content was found.

CONCLUSION: The method of powder infusion should be chosen if high concentration of antioxidants are sought. On the other hand, a high antioxidant capacity and a high phenol content correlate well with the empirically established (and widely publicised) capacity to treat respiratory infections.

Keywords: *Persea americana* (Lauraceae); powder; leaves; $ABTS^{\bullet+}$; Folin–Ciocalteu

INTRODUCTION

Antioxidants have been extensively employed in the food industry, mainly as preservatives. Crude extracts of fruits, herbs, vegetables and cereals, as well as derived products, are particularly rich in phenolic compounds, so they have been a focus of attention by industry, because they can retard oxidative degradation of lipids, for example, and thereby improve quality and nutritional value of lipid-containing foods.[1] Phenolic compounds have been commonly found in both edible and non-edible plants, in which they exhibit a multiplicity of biological effects, including anti-inflammatory capacity, cholesterol regulation, vascular problem healing and antioxidant activity. The latter activity lies in their capacity to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators.[2]

Further to such preservation features, antioxidants are now increasingly sought in the human diet because of their benefits upon health. In this regard, antioxidants are viewed as compounds that protect cells against oxidative stress, which might otherwise lead to cell damage.[3–6] Coronary heart diseases, ulcers, cancer and neurodegenerative diseases (e.g. Parkinson's and Alzheimer's), besides overall ageing, are but a few examples of diseases and conditions that can be prevented (or at least delayed) via regular and balanced inclusion of antioxidants in the human diet.[7,8]

There is a long history of medicinal properties ascribed to plants that grow wild in nature, and such plants still constitute a major source of pharmaceutical and healthcare products. The active roles of several herbal infusions in disease prevention (and even cure) have been attributed, at least in part, to antioxidant properties of their constituent (liposoluble) vitamins A and E, (water soluble) vitamin C, and several amphipathic molecules, which are broadly termed phenolics.[9] A whole range of plant-derived dietary supplements, phytochemicals and pro-vitamins, which that have been claimed to assist in maintaining good health and fighting disease, are now described as

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nutraceuticals, and are thus more and more widely used in the formulation of functional foods.

A few analytical methods have been developed and described at some length, in attempts to determine total antioxidant capacity, the choice of which depends on the purpose of each particular study. However, to date, no method has earned the consistent agreement of the whole scientific community, and it is often suggested that different methods should be considered for a more correct and deeper insight of a given plant matrix. In what concerns the antioxidant activity of medicinal plants, the most successful methods are probably 2,2-azinobis(3-ethylbenzothizoline-6-sulfonic acid) diammonium salt radical cation ($ABTS^{\bullet+}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC),[10–12] whereas for total phenolic compound content, the preferred method is likely Folin–Ciocalteu.

The objective of this research work was therefore to determine the total antioxidant capacity (via the $ABTS^{\bullet+}$ method) and the total phenol content (via the Folin–Ciocalteu method) of aqueous infusions of most of the medicinal plants traditionally used in Portugal. To our knowledge, this is the most comprehensive study performed to date on this topic. In order to assess the effect of the mode of preparation on the total antioxidant capacity and phenol content of the infusions, three alternative recipes were tested: infusion or boiling of the plant as such (or of its components, such as leaves or flowers), or infusion after milling the plant.

MATERIALS AND METHODS

Sample preparation

The 48 medicinal plants tested (Table 1) were a gift from ERVITAL (Castro Daire, Portugal). All such plants had been cultivated as organic products, and are currently sold associated with a number of assumed health claims. Each plant was used with the specifications normally available at the market (in the form of leaves, flowers, or a mixture of both), and was termed 'leaves'; after milling, the term 'powder' was used instead; in either case, it was employed to prepare an infusion, via addition of 110 mL of boiling water to 1 g of plant and/or powder; after 5 min (i.e. the time period typically used by the consumer), the extract was filtered through a 0.45 μm filter. For boiling (decoction), 110 mL of water was added to 1 g of plant; the mixture was heated until boiling, which was maintained for 5 min; afterwards, the extract was also filtered through a 0.45 μm filter.

Total antioxidant capacity

An improved ABTS-based method[10] was implemented. According to this technique, direct production of the (blue/green) $ABTS^{\bullet+}$ chromophore was achieved via reaction between ABTS and potassium persulfate; this method is able to quantify both water- and lipid-soluble antioxidants, as pure compounds or in crude extracts containing them.[10] Slight modifications introduced in the present research effort included the solvent solution used for $ABTS^{\bullet+}$ and the compound used as standard. In our version of this method, the cation $ABTS^{\bullet+}$ was diluted with ultra-pure water, and no differences were observed, irrespective of temperature, in the range 25–30 °C; this solvent possesses the advantage of a high stability, for both the stock and the dilute solutions. Trolox, used as a standard in the original method, was replaced in our case by ascorbic acid (99.0% pure, from Sigma-Aldrich, Steinheim, Germany) because: (1) it is widely used by the food industry; (2) results are at least as reproducible as those obtained with trolox; (3) preparation of the corresponding solutions is easier; and (4) the final solution exhibits a higher stability (as stressed above). Quantitative results (in g L^{-1} of ascorbic acid equivalent) were obtained through calibration curves produced using standard solutions of ascorbic acid. In this way, the final results generated were easier to interpret, and it was also easier to compare samples among them.

The $ABTS^{\bullet+}$ solution was prepared via addition, at 1:1 (v/v), of 7 mmol L^{-1} ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Sigma-Aldrich)) to 2.45 mmol L^{-1} potassium persulfate (Merk, Damstadt, Germany) solutions; the reaction took place in the dark for 16 h. In order to obtain an absorbance of 0.700 ± 0.020, at 734 nm, measured with an UV 1203 and an UV mini 1240 spectrophotometers (both from Shimadzu, Tokyo, Japan), the aforementioned $ABTS^{\bullet+}$ solution was duly diluted in ultra-pure water. For analysis of experimental samples, an accurate volume was used in order to obtain an inhibition percentage between 20 and 80%, by 6 min of reaction, with 1 mL of $ABTS^{\bullet+}$ solution; the average of three replicates was used as a datum point. The total antioxidant capacity was expressed as percentage of inhibition (PI), according to the equation $PI = (Abs_{ABTS\bullet+} - Abs_{sample})/Abs_{ABTS\bullet+}) \times 100$, where $Abs_{ABTS\bullet+}$ denotes the initial absorbance of diluted $ABTS^{\bullet+}$, and Abs_{sample} denotes the absorbance of the sample by 6 min of reaction. Using the calibration curve, previously prepared with ascorbic acid as standard, the final result was thus expressed as equivalent concentration of ascorbic acid (in g L^{-1}).

Total phenol content

The lumped concentration of phenolic compounds was determined as described elsewhere.[11] The chromophore development reaction is based on oxidation of polyphenols via Folin–Ciocalteu reagent, which is a mixture of phosphomolybdic and phosphotungstic acids, in a basic medium; the blue complex thus formed is assayed for absorbance at 750 nm, which is directly proportional to the total amount of polyphenols in the medium. To 0.5 mL of sample, 0.5 mL of Folin–Ciocalteu reagent (Merck), 10 mL of 75 g L^{-1} sodium carbonate (Sigma-Aldrich) and

Table 1. Medicinal plants employed traditionally in Portugal as aqueous infusions, including alternative denominations and widely accepted health claims

				Application in traditional medicine																	
Portuguese common name	English common name	Scientific name (family)	Part of plant used	Uric acid control	Diarr-hoea	Dig-estion	Liver	Res-piratory system	Anal-gesic	Diuretic	Wounds	Cholest-erol control	Dis-infectant	Anti-inflam-matory	In-somnia	Rheu-matism	Urinary system	High blood pressure	Vas-cular system	Gyna-eco-logical disease	Lax-ative
Abacateiro	Avocado	*Persea americana* (Lauraceae)	Leaves	X	X	X	X	X													
Agrimónia	Agrimony	*Agrimonia eupatoria* (Rosaceae)	Leaves	X				X	X	X	X										
Alcachofra	Artichoke	*Cynara cardunculus* (Asteraceae)	Leaves				X					X									
Alecrim	Rosemary	*Rosmarinus officinalis* (Lamiaceae)	Leaves				X		X				X								
Alfazema	Lavender	*Lavandula officinalis* (Lamiaceae)	Flowers					X	X				X	X	X	X					
Bétula	Birch	*Betula pendula* (Betulaceae)	Leaves	X						X		X				X	X				
Camomila	Chamomile	*Chamomilla* sp. (Asteraceae)	Flowers		X	X		X		X	X		X	X	X						
Camomila	Chamomile	*Matricaria chamomilla* (Asteraceae)	Flowers		X	X		X		X	X		X	X	X						
Carqueja	Winged Broom	*Chamaespartium tridentatum* (Leguminosae)	Flowers					X						X							
Cavalinha	Horsetail	*Equisetum arvense* (Equisetaceae)	Leaves			X				X											
Chá príncipe	Lemon grass	*Cymbopogon citratus* (Poaceae)	Leaves			X						X			X						
Coentros	Coriander	*Coriandrum sativum* (Apiaceae)	Mixture													X					
Equinácea	Echinacea	*Echinacea purpurea* (Asteraceae)	Leaves											X							
Estigmas de milho	Maize stigmas	*Zea mays* (Poaceae)	Flowers							X		X			X		X	X			

(*continued overleaf*)

Table 1. Continued

Portuguese common name	English common name	Scientific name (family)	Part of plant used	Application in traditional medicine																	
				Uric acid control	Diarr-hoea	Dig-estion	Liver	Res-piratory system	Anal-gesic	Diuretic	Wounds	Cholest-erol control	Dis-infectant	Anti-inflam-matory	In-somnia	Rheu-matism	Urinary system	High blood pressure	Vas-cular system	Gyna-eco-logical disease	Lax-ative
Estragão	Tarragon	*Artemisia dracunculus* (Asteraceae)	Leaves			X							X								
Eucalipto	Eucalyptus	*Eucalyptus globulus* (Myrtaceae)	Leaves					X					X	X							
Fel da terra	Red centaury	*Erythraea centaurium* (Gentianaceae)	Mixture			X											X		X		
Flor de sabugueiro	Black elder flowers	*Sambucus nigra* (Caprifoliaceae)	Flowers					X						X	X						
Flor de milefólio	Yarrow	*Achillea millefolium* (Asteraceae)	Flowers	X	X	X							X							X	
Framboeseiro	Raspberry	*Rubus idaeus* (Rosaceae)	Leaves			X				X				X			X		X	X	X
Freixo	Ash	*Fraxinus* (Oleaceae)	Leaves	X						X		X		X		X	X		X		X
Funcho	Fennel	*Foeniculum vulgare* (Apiaceae)	Mixture			X															
Giesta branca	White Spanish broom	*Cytisus multiflorus* (Fabaceae)	Flowers	X						X							X	X			
Hipericão	St John's wort	*Hypericum perforatum* (Clusiaceae)	Leaves		X		X						X		X		X			X	
Hipericão do Gerês	Sweet amber	*Hypericum androsaemum* (Clusiaceae)	Leaves			X	X				X						X				
Hissopo	Hyssop	*Hyssopus officinalis* (Lamiaceae)	Leaves			X	X	X					X						X		
Hortelã-comum	Spearmint	*Mentha spicata* (Lamiaceae)	Leaves		X	X	X														
Hortelã-pimenta	Peppermint	*Mentha piperita* (Lamiaceae)	Leaves		X	X	X	X													
Levístico	Lovage	*Levisticum officinale* (Apiaceae)	Leaves			X			X												

Loureiro	Laurel	*Laurus nobilis* (Lauraceae)	Leaves			X																X
Lúcia-lima	Lemon verbena	*Lippia citriodora* (Verbenaceae)	Leaves			X								X	X							
Macela	Wild camomile	*Chamaemelum nobile* (Asteraceae)	Flowers		X	X			X	X				X								
Malvas	Dwarf mallow	*Malva silvestris* (Malvaceae)	Leaves		X				X					X								
Manjericão	Sweet basil	*Ocimum basilicum* (Lamiaceae)	Leaves			X				X					X							
Murta	Myrtle	*Myrtus communis* (Myrtaceae)	Leaves					X										X				
Nogueira	Walnut-tree	*Juglans regia* (Juglandaceae)	Leaves		X			X				X	X	X						X		
Oliveira	Olive leaf	*Olea europaea* (Oleaceae)	Leaves																X			
Poejo	European pennyroyal	*Mentha pulegium* (Lamiacea)	Leaves			X		X						X	X							X
Rosmaninho	French lavender	*Lavandula stoechas* (Lamiacea)	Flowers																	X		
Salva	Sage	*Salvia sp.* (Lamiacea)	Leaves		X	X						X		X	X					X		
Segurelha	Savory	*Satureja montana* (Lamiacea)	Leaves		X	X						X				X						
Tília	Linden tree	*Tilia cordata* (Tiliaceae)	Mixture			X	X			X				X	X							
Tomilho	Thyme	*Thymus vulgaris* (Lamiacea)	Leaves			X		X				X			X	X						
Tomilho-eucalipto	Spanish wood Marjoram	*Thymus mastichina* (Lamiacea)	Leaves					X				X			X							
Tomilho-limão	Lemon thyme	*Thymus citriodorus* (Lamiacea)	Leaves			X		X							X							
Ulmária	Queen-of-the meadow	*Filipendula ulmaria* (Rosaceae)	Leaves	X	X					X	X						X					
Urtiga	Nettles	*Urtica dioica* (Urticaceae)	Leaves		X													X		X		
Urze	Heath	*Calluna vulgaris* (Ericaceae)	Leaves								X				X			X				

water were added up to the final volume of 25 mL. Absorbance at 750 nm was measured on a Heλios α spectrophotometer (Unicam, Cambridge, UK). Gallic acid was used as standard to prepare calibration curves in the ranges 4–80 and 20–400 mg L^{-1}. The total phenol content was reported as gallic acid equivalent (C, in g L^{-1}), using the expression $C = (Abs_{sample} - 0.0201)/2.1456$, where Abs_{sample} denotes absorbance of the sample at 1 h of reaction. The Pearson correlation coefficient of the above fit was 0.9991.

Statistical analyses

Analysis of variance (ANOVA)[12] was applied to all experimental results produced, in attempts to assess the effects of the main parameters, viz. type of extraction and analytical method. Tukey's test was also applied to all experimental results, with the goal of pinpointing statistically significant differences (at the 5% level), among all possible pairs within the extraction treatment. Principal component analysis (PCA) was also applied to all experimental results, to simultaneously assess correlations among analytical variables (viz. $ABTS^{\bullet+}$ and Folin–Ciocalteu) and extraction methods. Given the heteroschedasticity of our experimental data regarding both analytical methods, a logarithmic transform had to be applied prior to statistical analysis (hence giving rise to the negative values in the figures and tables). Correlation analysis was applied encompassing the nominal data versus the quantitative data (i.e. effect on diseases, and total antioxidant capacity and total phenolic content, respectively). All analyses were conducted using SPSS 13.0.0 software (SPSS, Chicago IL, USA).

RESULTS AND DISCUSSION

A summary of the traditional medicine applications of all plants studied (which is a part of the cultural heritage of the local producers, and is comprehensively and systematically conveyed by the supplier) is given in Table 1, as a matrix of occurrences, which encompass digestive, urinary, reproductive, cardiovascular and respiratory disorders, as well as healing wounds, fighting inflammation and suppressing pain. The data produced pertaining to the antioxidant activity and total phenol content of the 48 medicinal plants tested are presented in Table 2, and major differences can easily be observed among them. In terms of antioxidant activity, the ascorbic acid equivalent concentration of herbal plant extracts ranged from $1.4280 \pm 0.1261\,g\,L^{-1}$ for avocado (*Persea americana*, Lauraceae) obtained by powder infusion, down to $0.0027 \pm 0.0012\,g\,L^{-1}$ for olive leaf (*Olea europaea*, Oleaceae) obtained by leaf infusion. In terms of total phenolic content, the gallic acid equivalent concentration ranged from $0.5541 \pm 0.0289\,g\,L^{-1}$ for avocado obtained by powder infusion, down to $0.0053 \pm 0.0014\,g\,L^{-1}$ for olive leaf, obtained by leaf boiling. In general, the highest antioxidant activity and phenol content were observed for avocado, agrimony (*Agrimony eupatoria*, Rosaceae), eucalyptus (*Eucalyptus globules*, Myrtaceae), yarrow (*Achillea millefolium*, Asteraceae), myrtle (*Myrtus communis*, Myrtaceae), thyme (*Thymus vulgaris*, Lamiacea) and heath (*Calluna vulgaris*, Ericaceae).

Avocado yields unique and particularly interesting results, as little information has so far been made available on this plant. On the other hand, agrimony has been studied but only in terms of seed extracts, which have shown a good antimicrobial effect, probably as a consequence of its good antioxidant activity due to coumarins, flavonois, tannims and terpenoids;[13] similar results were also described for yarrow,[14] but in this case the major components found in methanol extracts were eucalyptol, camphor, α-terpineol, β-pinene and borneol.[15] Previous studies encompassing eucalyptus[16] revealed its richness in ellagic acid rhamnosides, which are compounds that inhibit lipid peroxidation, as observed in rat liver microsomes. From the array of herbal and medicinal plants tested, 29 constituted topics of previous works, particularly rosemary (*Rosmarinus officinalis*, Lamiaceae)[15,17–32] and sage (*Salvia officinalis*, Lamiacea).[33–38]

From the data in Table 2, it can be concluded that thyme exhibits high values of total antioxidant capacity ($1.2931 \pm 0.2100\,g\,L^{-1}$ of ascorbic acid equivalent), as well as total phenol content ($0.5114 \pm 0.0638\,g\,L^{-1}$ of gallic acid equivalent), both obtained for powder infusion. Rosemary and sage did not exhibit significantly higher antioxidant activity and phenol content than the other plants considered; however, both have been described[17] to possess an important hepaprotective effect in rats. In rosemary, an important antioxidant, carnosol, was claimed[15,18] to inhibit invasion by B16/F10 mouse melanoma cells. These results thus confirm that antioxidant capacity *per se* may not be enough, if specific health effects are sought.

Both the minimum and the maximum values of the antioxidant activity and the polyphenol content of the medicinal plants tested are given in Table 3, together with their average and standard deviation descriptors. The ranges of variation within plants are indeed substantial.

As expected,[39] the results pertaining to total antioxidant capacity and total phenolic content show a strong linear positive correlation with each other (Pearson coefficient of 0.838, $P < 0.0001$), irrespective of extraction method, as concluded from ANOVA (see Table 4). From a statistical point of view, both analytical variables showed high discriminative power to distinguish between medicinal plant extracts. One-way ANOVA was used in order to check whether the average values obtained for each analytical method could be considered different or not, for all extraction methods.[12] (This statistical analysis was possible because data obtained as values, for each experiment, presented normal distributions, as checked by the Shapiro–Wilk test; and homoschedasticity of variances, as checked by

Table 2. Total antioxidant capacity and total phenol content (average ± standard deviation) of medicinal plant extracts

		ABTS$^{\bullet+}$ (g L^{-1} ascorbic acid equivalent)			Folin–Ciocalteu (g L^{-1} gallic acid equivalent)		
Portuguese common name	English common name	Powder infusion	Leaf infusion	Leaf boiling	Powder infusion	Leaf infusion	Leaf boiling
Abacateiro	Avocado	1.428 ± 0.126	0.039 ± 0.002	0.126 ± 0.049	0.554 ± 0.029	0.024 ± 0.009	0.123 ± 0.048
Agrimónia	Agrimony	0.609 ± 0.039	0.143 ± 0.014	0.443 ± 0.036	0.308 ± 0.007	0.117 ± 0.014	0.242 ± 0.026
Alcachofra	Artichoke	0.127 ± 0.007	0.134 ± 0.074	0.371 ± 0.018	0.220 ± 0.003	0.160 ± 0.073	0.205 ± 0.014
Alecrim	Rosemary	0.303 ± 0.041	0.009 ± 0.000	0.036 ± 0.015	0.358 ± 0.006	0.025 ± 0.047	0.030 ± 0.009
Alfazema	Lavender	0.291 ± 0.020	0.117 ± 0.004	0.468 ± 0.020	0.220 ± 0.021	0.109 ± 0.004	0.217 ± 0.002
Bétula	Birch	0.145 ± 0.022	0.069 ± 0.005	0.138 ± 0.012	0.128 ± 0.018	0.039 ± 0.004	0.185 ± 0.018
Camomila	Chamomile	0.265 ± 0.009	0.077 ± 0.006	0.160 ± 0.048	0.196 ± 0.011	0.070 ± 0.011	0.171 ± 0.035
Camomila	Chamomile	0.291 ± 0.010	0.148 ± 0.016	0.259 ± 0.017	0.228 ± 0.006	0.140 ± 0.010	0.206 ± 0.006
Carqueja	Winged Broom	0.164 ± 0.036	0.057 ± 0.025	0.260 ± 0.030	0.308 ± 0.004	0.130 ± 0.026	0.265 ± 0.019
Cavalinha	Horsetail	0.336 ± 0.005	0.008 ± 0.001	0.285 ± 0.017	0.214 ± 0.044	0.109 ± 0.024	0.139 ± 0.008
Chá príncipe	Lemon grass	0.077 ± 0.012	0.055 ± 0.016	0.224 ± 0.078	0.098 ± 0.015	0.066 ± 0.020	0.079 ± 0.027
Coentros	Coriander	0.107 ± 0.007	0.027 ± 0.005	0.127 ± 0.005	0.119 ± 0.014	0.023 ± 0.005	0.104 ± 0.005
Equinácea	Echinacea	0.085 ± 0.012	0.047 ± 0.012	0.061 ± 0.009	0.108 ± 0.019	0.062 ± 0.007	0.055 ± 0.024
Estigmas de milho	Maize stigmas	0.045 ± 0.019	0.031 ± 0.007	0.115 ± 0.064	0.053 ± 0.021	0.030 ± 0.007	0.103 ± 0.033
Estragão	Tarragon	0.299 ± 0.101	0.076 ± 0.019	0.321 ± 0.068	0.301 ± 0.007	0.089 ± 0.006	0.241 ± 0.043
Eucalipto	Eucalyptus	1.350 ± 0.117	0.027 ± 0.016	0.136 ± 0.010	0.430 ± 0.017	0.045 ± 0.032	0.089 ± 0.012
Fel da terra	Red centaury	0.078 ± 0.008	0.040 ± 0.013	0.093 ± 0.004	0.108 ± 0.023	0.029 ± 0.012	0.102 ± 0.002
Flor de sabugueiro	Black elder flowers	0.043 ± 0.003	0.011 ± 0.001	0.173 ± 0.014	0.040 ± 0.007	0.017 ± 0.010	0.294 ± 0.038
Flor de milefólio	Yarrow	1.074 ± 0.061	0.323 ± 0.083	0.240 ± 0.006	0.431 ± 0.047	0.178 ± 0.089	0.132 ± 0.003
Framboeseiro	Raspberry	0.077 ± 0.043	0.058 ± 0.017	0.173 ± 0.001	0.269 ± 0.019	0.144 ± 0.022	0.488 ± 0.002
Freixo	Ash	0.178 ± 0.006	0.029 ± 0.013	0.098 ± 0.005	0.212 ± 0.007	0.028 ± 0.016	0.111 ± 0.012
Funcho	Fennel	0.179 ± 0.055	0.155 ± 0.001	0.134 ± 0.018	0.232 ± 0.088	0.216 ± 0.030	0.118 ± 0.017
Giesta branca	White Spanish broom	0.092 ± 0.025	0.068 ± 0.018	0.176 ± 0.007	0.117 ± 0.020	0.015 ± 0.001	0.238 ± 0.014
Hipericão	St John's wort	0.252 ± 0.043	0.074 ± 0.013	0.152 ± 0.027	0.204 ± 0.030	0.057 ± 0.019	0.160 ± 0.009
Hipericão do Gerês	Sweet amber	0.145 ± 0.058	0.113 ± 0.014	0.374 ± 0.022	0.410 ± 0.004	0.205 ± 0.064	0.348 ± 0.054
Hissopo	Hyssop	0.150 ± 0.010	0.036 ± 0.007	0.104 ± 0.013	0.170 ± 0.029	0.201 ± 0.015	0.124 ± 0.021
Hortelã-comum	Spearmint	0.144 ± 0.073	0.099 ± 0.048	0.355 ± 0.071	0.355 ± 0.030	0.103 ± 0.055	0.295 ± 0.006
Hortelã-pimenta	Peppermint	0.403 ± 0.105	0.320 ± 0.076	0.537 ± 0.117	0.308 ± 0.109	0.246 ± 0.030	0.291 ± 0.185
Levístico	Lovage	0.092 ± 0.016	0.031 ± 0.003	0.088 ± 0.006	0.114 ± 0.026	0.027 ± 0.007	0.123 ± 0.015
Loureiro	Laurel	0.205 ± 0.044	0.005 ± 0.001	0.030 ± 0.009	0.167 ± 0.037	0.010 ± 0.002	0.024 ± 0.010
Lúcia-lima	Lemon verbena	0.048 ± 0.004	0.043 ± 0.005	0.052 ± 0.003	0.077 ± 0.043	0.067 ± 0.007	0.064 ± 0.005
Macela	Wild camomile	0.149 ± 0.047	0.045 ± 0.016	0.086 ± 0.003	0.180 ± 0.040	0.030 ± 0.017	0.080 ± 0.003
Malvas	Dwarf mallow	0.135 ± 0.046	0.061 ± 0.007	0.090 ± 0.001	0.098 ± 0.010	0.072 ± 0.014	0.073 ± 0.002
Manjericão	Sweet basil	0.179 ± 0.012	0.071 ± 0.014	0.156 ± 0.038	0.155 ± 0.008	0.032 ± 0.017	0.161 ± 0.019
Murta	Myrtle	1.280 ± 0.151	0.006 ± 0.001	0.088 ± 0.007	0.494 ± 0.022	0.011 ± 0.001	0.057 ± 0.008
Nogueira	Walnut-tree	0.282 ± 0.039	0.011 ± 0.051	0.173 ± 0.050	0.291 ± 0.031	0.056 ± 0.031	0.242 ± 0.050
Oliveira	Olive leaf	0.275 ± 0.098	0.003 ± 0.001	0.018 ± 0.001	0.274 ± 0.015	0.007 ± 0.004	0.005 ± 0.001
Poejo	European pennyroyal	0.174 ± 0.056	0.100 ± 0.001	0.124 ± 0.025	0.190 ± 0.013	0.072 ± 0.021	0.182 ± 0.020
Rosmaninho	French lavender	0.150 ± 0.018	0.105 ± 0.014	0.222 ± 0.009	0.144 ± 0.003	0.385 ± 0.166	0.218 ± 0.020
Salva	Sage	0.144 ± 0.030	0.172 ± 0.044	0.315 ± 0.055	0.432 ± 0.005	0.202 ± 0.005	0.292 ± 0.034
Segurelha	Savory	0.419 ± 0.206	0.062 ± 0.009	0.202 ± 0.011	0.260 ± 0.067	0.089 ± 0.047	0.167 ± 0.018
Tília	Linden tree	0.422 ± 0.045	0.066 ± 0.010	0.193 ± 0.035	0.233 ± 0.055	0.040 ± 0.027	0.126 ± 0.027
Tomilho	Thyme	1.293 ± 0.210	0.456 ± 0.048	0.652 ± 0.055	0.511 ± 0.064	0.286 ± 0.045	0.508 ± 0.054
Tomilho-eucalipto	Spanish wood Marjoram	0.346 ± 0.041	0.172 ± 0.093	0.374 ± 0.028	0.287 ± 0.026	0.152 ± 0.044	0.252 ± 0.018
Tomilho-limão	Lemon thyme	0.265 ± 0.048	0.094 ± 0.015	0.162 ± 0.013	0.254 ± 0.007	0.103 ± 0.010	0.164 ± 0.007
Ulmária	Queen-of-the meadow	0.097 ± 0.026	0.046 ± 0.016	0.091 ± 0.004	0.117 ± 0.002	0.064 ± 0.015	0.087 ± 0.003
Urtiga	Nettles	0.083 ± 0.043	0.113 ± 0.035	0.113 ± 0.009	0.149 ± 0.035	0.163 ± 0.029	0.141 ± 0.019
Urze	Heath	0.590 ± 0.027	0.025 ± 0.008	0.091 ± 0.009	0.360 ± 0.051	0.014 ± 0.008	0.057 ± 0.011

the Levene test). The information in Tables 3 and 4 indicates, on the other hand, that levels of active compounds (as assessed by using ABTS$^{\bullet+}$ and Folin–Ciocalteu) are statistically different (at the 5% level) between extraction methods. Furthermore, the results obtained show that powder infusion is the best method for obtaining the most active and richest extracts in terms of total phenol content,

Table 3. Statistical descriptives of the $ABTS^{\bullet+}$ and Folin–Ciocalteu methods ($n = 48$)

Method	Preparation	Mean	Standard error	Minimum	Maximum
$ABTS^{\bullet+}$	Powder infusion	0.320472	0.0517185	0.0426	1.4280
	Leaf infusion	0.084874	0.0126753	0.0027	0.4560
	Leaf boiling	0.197048	0.0199205	0.0180	0.6520
	Total	0.200798	0.0204661	0.0027	1.4280
Folin-Ciocalteu	Powder infusion	0.239267	0.0180990	0.0397	0.5541
	Leaf infusion	0.094907	0.0118599	0.0073	0.3851
	Leaf boiling	0.170293	0.0154545	0.0053	0.5075
	Total	0.168156	0.0100881	0.0053	0.5541

Table 4. Means for groups, in homogeneous subsets of each of the three extraction methods, obtained from Tukey's test (with 95% confidence) for each of the two analytical methods

		$ABTS^{\bullet+}$ method			Folin–Ciocalteu method		
Preparation	*n*	1	2	3	1	2	3
Leaf infusion	48	0.085	–	–	0.239	–	–
Leaf boiling	48	–	0.095	–	–	0.197	–
Powder infusion	48	–	–	0.170	–	–	0.320
Significance	–	1.000	1.000	1.000	1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed, using the harmonic mean sample size (48).

and also in terms of antioxidant capacity. Hence, an increase of specific area seems to significantly influence the extraction of antioxidants and phenols: this was somewhat expected, because of the much higher specific area of the former that is available for mass transfer, and which, apparently, is more effective than a higher temperature upon extraction. This point is further complemented by the observation that a low temperature combined with a low degree of division of the feedstock (leaf infusion) yields the lowest degree of extraction.

In figure 1, it is shown that, for the majority of medicinal plants tested (about 30), the antioxidant capacity depends on the extraction method. Two distinct groups can be identified: one in which the highest dependence is associated with powder infusion (A), and one in which the highest dependence is observed for leaves (B). From the plants that constitute these groups, those that show higher antioxidant capacity and total phenol content belong to the former group (A) and include avocado, winged broom (*Chamaespartium tridentatum,* Leguminosae), lemon grass (*Cymbopogon citrates*, Poaceae), maize stigmae (*Zea mays*, Poaceae), black elder flowers (*Sambucus nigra*, Caprifoliaceae) and sweet amber (*Hypericum androsaemum*, Clusiaceae). The latter group (B) encompasses seven plants: agrimony, lavender (*Lavandula officinalis*, Lamiaceae), horsetail (*Equisetum arvense*, Equisetaceae), echinacea (*Echinacea purpurea*, Asteraceae), tarragon (*Artemisia dracundulus*, Asteraceae), yarrow (*Achillea millefolium*, Asteraceae) and white Spanish broom (*Cytisus multiflorus*, Fabaceae). However, among all the plants studied, it can be seen that some of them, which can be removed from any of those groups, exhibit more interesting

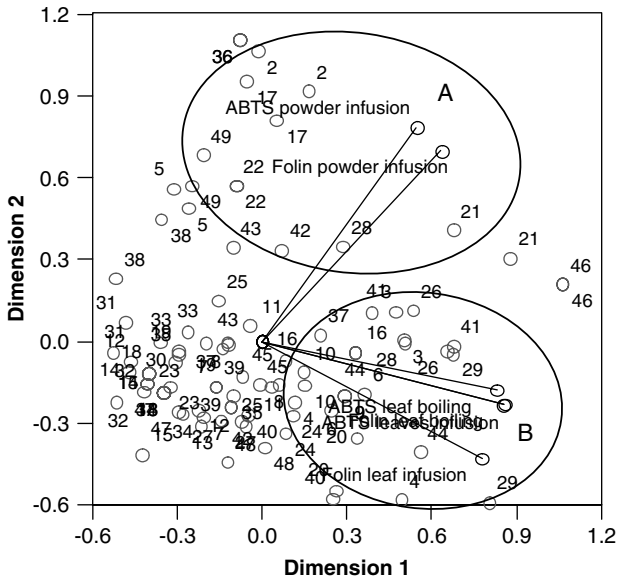

Figure 1. Distribution of extracts according to oxidation method. Variable principal normalisation, with objects (○) labelled by common name, and including component loadings (——).

values of total antioxidant capacity and total phenol content, e.g. eucalyptus, myrtle, thyme and heath.

To take full advantage of the data available elsewhere and generated here, a tentative correlation was sought between total antioxidant capacity (and total phenol content) with empirical health claims. The results are given in Table 5. Powder infusion extracts only were considered, because these data correlate best with the actual inventory of those plants in terms of antioxidant activity and polyphenol content. It is remarkable that besides the high correlation between antioxidant capacity and total phenol content, there is also a statistically significant correlation (at

Table 5. Pearson correlation between traditional medicine application, and total antioxidant capacity and total phenol content of the powder infusion of the 48 plants studied

	Pearson correlation	
Application in traditional medicine	$ABTS^{\bullet+}$	Folin-Ciocalteu
Uric acid control	0.230	0.092
Diarrhoea	0.088	0.167
Digestion	0.041	0.157
Liver	0.077	0.283
Respiratory system	0.440**	0.342*
Analgesic	−0.024	−0.022
Diuretic	−0.163	−0.276
Wounds	0.107	0.294
Cholesterol control	−0.190	−0.220
Disinfectant	0.331*	0.283
Anti-inflamatory	−0.006	−0.012
Insomnia	−0.178	−0.236
Rheumatism	−0.151	−0.220
Urinary system	−0.078	−0.051
High blood pressure	−0.133	−0.190
Vascular system	−0.221	−0.062
Gynaecological diseases	0.034	0.036
Laxative	−0.084	−0.029

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

Table 6. Plants with the highest antioxidant capacity and phenol content

	Concentration of equivalent ascorbic or gallic acid (g g^{-1} powder)	
Plant	Ascorbic acid	Gallic acid
Avocado	0.157	0.061
Agrimony	0.067	0.034
Eucalyptus	0.149	0.047
Yarrow	0.118	0.047
Myrtle	0.141	0.054
Thyme	0.142	0.056
Heath	0.065	0.040

the 1% level) with positive effects upon respiratory affections. However, further trials are required to determine whether such clinical benefits are related to the observed overall antioxidant capacity as such, or to some specific phenolic compound(s) that account for it.

CONCLUSIONS

The mode of preparation of medicinal plant extracts affects the extent of extraction of antioxidants: plant infusion in the form of powder should be selected, as it is the most effective of the modes tested. It is noteworthy that this is already the most commonly form of preparation of associated drinks in Portugal, at home, public restaurants and tea houses. This apparently results from the fact that a specific area of the feedstock is a more relevant factor with regard to extraction than is the temperature of the solvent.

The highest antioxidant capacity and phenolic content are observed for avocado followed by agrimony, eucalyptus, yarrow, myrtle, thyme and heath (Table 6).

The antioxidant capacity detected is directly associated to the phenolic content, with a high correlation coefficient. Despite the fact that antioxidant capacity and phenol content are dependent on the extraction method for many plants, there is some statistical evidence that the former is caused by the latter. Finally, the high values of antioxidant capacity and phenol content are statistically related with an effective performance in treating respiratory problems, confirming evidence acquired by traditional knowledge.

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