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The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal

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Abstract

Essential oil, ethanolic extract and decoction of 10 plant species from interior Portugal were analyzed for their activity towards acetylcholinesterase (AChE) enzyme and their antioxidant activity. Of these, *Melissa officinalis, Paronychia argentea, Sanguisorba minor*, *Hypericum undulatum* and *Malva silvestris* are used in herbal medicine, *Laurus nobilis* and *Mentha suaveolens* as condiments, and *Salvia officinalis*, *Lavandula angustifolia* and *Lavandula pedunculata* also as aromatics. *Melissa officinalis* and *Mentha suaveolens* showed AChE inhibitory capacity higher then 50% in the essential oil fraction. *Laurus nobilis, Hypericum undulatum*, and *Sanguisorba minor* showed a high inhibition value of AChE in the ethanolic fraction, 64% (1 mg ml^{-1}) 68% (0.5 mg ml^{-1}), and 78% (1 mg ml^{-1}), respectively. Higher values of AChE inhibitory activity were found using decoctions of *Lavandula pedunculata*, *Mentha suaveolens* and *Hypericum undulatum*, 68, 69 and 82% (at a concentration of 5 mg dry plant ml^{-1} of assay), respectively. The free radical scavenger activity was higher for the polar extracts. In the water extracts most of the plants showed values around 90%. When antioxidant activity was measured with the β-carotene-linoleic acid assay high activity (65–95%) was also found in the water extracts. *Hypericum undulatum*, *Melissa officinalis* and *Laurus nobilis* showed both high AChE inhibitory capacity and antioxidant activity.

Keywords: Acetylcholinesterase; Alzheimer; Antioxidant; Essential oil; Ethanolic extract; Water extract

1. Introduction

Alzheimer's disease (AD) is frequent in elderly people, as a result of malfunctioning of different biochemical pathways. There are several routes to tackle this problem (Perry et al., 2003; Citron, 2004), although the one that has been most successful so far is the "cholinergic hypothesis". The drugs approved for the AD therapy act by counteracting the acetylcholine deficit, that is, they try to enhance the acetylcholine level in the brain (Heinrich and Teoh, 2004). Acetylcholine is involved in the signal transfer in the synapses. After being delivered in the synapses, acetylcholine is hydrolyzed giving choline and acetyl group in a reaction catalyzed by the enzyme acetylcholinesterase (Voet and Voet, 1995). The molecular basis of the Alzheimer drugs used so far, take advantage of their action as acetylcholinesterase inhibitors (Heinrich and Teoh, 2004). Some of the drugs approved for therapeutic use show hepatotoxicity (Knapp et al., 1994), consequently there have been a continuous search for new drugs. Plants that show some therapeutic effect have been used for a long time. Galanthamine, an alkaloid from snowdrop, was recently approved for use in Alzheimer therapy (Ingkaninan et al., 2003; Heinrich and Teoh, 2004). There has been a lot of research on the biological effect of plants traditionally used either in infusions or in traditional remedies as acetylcholinesterase inhibitors in vitro and also as memory enhancers in vivo (Perry et al., 2000; Ingkaninan et al., 2003; Tildesley et al., 2003; Heinrich and Teoh, 2004). These studies are carried out in order to find new molecules or a group of molecules that can be used in the therapy without the toxicity of the synthesized chemical compounds.

Recent studies (Stuchbury and Munch, 2005) have pointed out that AD is associated with inflammatory processes. β-Amiloid peptides contained in the senile plaques found in AD

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Table 1
Name and folk use of plants studied

Plant				Ethnobotanical information	
Systematic name	Common name	Portuguese local name	Part(s) used	Troubles treated/use	Preparation/administration
Hypericum undulatum	Wavy St. John Wort	Erva de São João[a]	Flowers	–[a]	Decoction/oral
Laurus nobilis	Laurel	Louro (loureiro)	Leaf	Condiment[b], influenza, bronchitis, digestive problems, menstruation, insomnia[c]	Meat dishes, stews, rice, infusion/oral
Lavandula angustifolia	Lavender	Alfazema	Flowered aerial parts, flowers	Anxiety, insomnia, anorexia[d], bronchitis, cough, nerves, rheumatism, heart disturbance[c]	Infusion/oral
Lavandula pedunculata	Butterfly lavender	Rosmaninho	Flowered aerial parts, flowers	Anxiety, insomnia, anorexia[d], Tonic, cough, bronchitis[c]	Infusion /oral
Malva silvestris	Mallow	Malva	Leaf Flower and/or leaf	Inflammation/infection[b] Constipation, antihypertensive[e], Constipation, obesity, digestive troubles, bladder and prostate ailments[c]	Decoction/external or oral Infusion/oral
Melissa officinalis	Lemon balm	Erva cidreira	Aerial part Whole plant Leaf[c]	Malaise[b] Nerves, insomnia, loss of memory[c] Sedative, digestive, analgesic, intestinal anti-inflammatory, hepatic protector, for sea-sickness, for renal and gall-bladder ailments[e]	Decoction/oral Infusion/oral Infusion/oral
Mentha suaveolens	Apple mint	Mentrasto	Aerial part	Condiment[b], influenza, colds, cough[c]	Soups, stews, decoction/oral
Paronychia argentea	–	Erva prata	Aerial part	Gastric analgesic, bladder and prostate ailments, abdominal ailments, stomach ulcers[e,c]	Infusion/oral
Salvia officinalis	Sage	Salva		Condiment, Anorexia, flatulence, *climacterium,* menopause, nerves, anti-impotence[d,c]	Meat dishes, stews, Infusion, tincture/oral
Sanguisorba minor	Little burnet	Pimpinela	Aerial part	Conjunctivitis, fever[b], diarrhoea, abdominal ailments[c]	Bath after boiling/external, infusion, tincture/external

[a] *Hypericum undulatum* is very similar, for a non expert, to *H. perforatum* and is given the same name "erva de S. João" or "hipericão" (personal information from Dr Ana Margarida Francisco). It is used for the treatment of the same troubles as *H. perforatum*: migraine, renal antispasmodic, hepatic protector, for bladder and gall bladder ailments, intestinal-inflammatory.
[b] Camejo-Rodrigues et al., 2003.
[c] Salgueiro, 2004.
[d] Proença da Cunha (2003).
[e] Novais et al., 2004.

brain can induce these inflammatory processes in which radical oxygen species (ROS) are liberated, among other components (Vina et al., 2004; Stuchbury and Munch, 2005). ROS are able to damage cellular constituents and act as secondary messenger in inflammation. Antioxidants can scavenge ROS and can also attenuate inflammation pathways. The use of antioxidants may be useful in the treatment of AD (Calabrese et al., 2003; Gibson and Huang, 2005).

Until three decades ago Portugal was a less developed country and, especially in the mountains localized in the interior, the populations had no easy access to primary health care and herbal medicines were very popular (Camejo-Rodrigues et al., 2003; Novais et al., 2004).

In the present work 10 plants used in the interior of Portugal for the treatment of different ailments and/or used as condiments, were collected in the country by the elderly. Most of the plants studied are used to treat "nerves" or related problems like anxiety, insomnia, anorexia or sexual impotency. Some are also used to treat other ailments. Table 1 contains a summary of folk uses. Three different extracts from the following plant species were searched for acetylcholinesterase inhibitory activity and also antioxidant capacity: *Melissa officinalis* L. (Lamiaceae) (lemon balm, *port.* erva cidreira), *Paronychia argentea* Lam. (Caryophyllaceae) (*port.* erva prata), *Sanguisorba minor* Scop. (Rosaceae) (little burnet, *port.* pimpinela), *Hypericum undulatum* Shoubs. Ex Willd (Hypericaceae) (wavy St. John's Wort, *port.* hipericão or erva de São João), *Malva silvestris* L. (Malvaceae) (mallow, *port.* malva), *Laurus nobilis* L. (Lauraceae) (laurel, *port.* louro), *Mentha suaveolens* Ehrh. (Lamiaceae) (apple mint, *port.* mentrasto), *Salvia officinalis* L. (Lamiaceae) (sage, *port*. salva), *Lavandula angustifolia* Miller (Lamiaceae) (lavender, *port.* alfazema) and *Lavandula pedunculata* (Miller) Cav. (Lamiaceae) (butterfly lavender, *port.* rosmaninho). It is known that many plants that have medicinal value are used as condiment or aromatic and the inclusion of this kind of plants in this study may determine their ability as functional foods or even pharmafoods (Hardy, 2000).

Essential oil, ethanolic extract and a decoction of the plants referred above were analyzed for acetylcholinesterase (E.C.3.1.1.7.) inhibitory capacity and their ability to function as antioxidants.

2. Material and methods

2.1. Plant material

Melissa officinalis (LISU 204116), *Paronychia argentea* (LISU 204110), *Sanguisorba minor* (LISU 204115), *Hypericum undulatum* (LISU 204117), *Malva silvestris* (LISU 204112), *Laurus nobilis* (LISU 204114), *Mentha suaveolens* (LISU 204111), *Lavandula pedunculata* (LISU 204107), were collected in the region of Fundão, Beira Interior (eastern Portugal), during the summer of 2004, except *Salvia officinalis* and *Lavandula angustifolia*, which were purchased at a local herbalist. Plant material was authenticated by Dr. Ana Margarida Francisco (research team of Professor Lia Ascenção, Centro de Biotecnologia Vegetal, DBV), Faculty of Science University of Lisbon, where voucher specimens have been deposited.

Table 2
Mass of dried plant (mg) necessary to obtain 1 mg of essential oil and ethanolic extracts

Plants	mg plant/mg essential oil	mg plant/mg ethanolic extract
Hypericum undulatum	581.4	7.09
Laurus nobilis	217.4	4.03
Lavandula angustifolia	216.5	7.47
Lavandula pedunculata	216.5	10.64
Malva silvestris	2666.7	16.13
Melissa officinalis	1013.2	12.82
Mentha suaveolens	166.9	23.81
Paronychia argentea	38.5	20
Salvia officinalis	89.9	23.26
Sanguisorba minor	212.8	12.66

2.2. Preparation of the extracts

Plant material was dried in the dark and ground to a powder to obtain essential oils and ethanolic extracts. It was used only 12–30 g (depending on the material) of the aerial parts except in the case of *Laurus nobilis* in which only the leaves were used.

Essential oils were obtained by hydrodistillation of the plant material until 200 ml of the water-oil layer was obtained. The aqueous layer was extracted with *n*-pentane and the organic solvent was removed by vacuum distillation at room temperature.

Ethanolic extracts were obtained by extracting the plant material three times at room temperature, and the solvent removed by vacuum distillation.

Table 2 presents the quantity (mg) of plant material necessary to obtain 1 mg of essential oil and ethanolic extract.

Decoctions were prepared by boiling 5 g of dried plant material, broken into small pieces, in 100 ml of distilled water for 20 min. Solutions were filtered. Aliquots of 1 ml were frozen and used when necessary for the enzymatic tests.

2.3. Chemicals

Acetylcholinesterase (AChE) type VI-S, from electric eel 349 U/mg solid, 411 U/mg protein, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide (AChI), Tris[hydroxymethyl]aminomethane (Tris buffer), dimethylsulfoxide (DMSO), Tween 40, linoleic acid, β-carotene, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-tert-butyl-4- hydroxytoluene (BHT) were bought from Sigma.

2.4. Acetylcholinesterase activity

The enzymatic activity was measured using an adaptation of the method described in Ingkaninan et al. (2003). 500 μl of DTNB 3 mM, 100 μl of AChI 15 mM, 275 μl of Tris–HCl buffer 50 mM, pH 8 and 100 μl of each ethanolic extract, decoction or essential oil fraction were dissolved in ethanol, water or DMSO, respectively, and were added to a 1 ml cuvette. This

cuvette was used as blank. In the reaction cuvette, 25 μl of buffer were replaced by the same volume of an enzyme solution containing 0.28 U ml^{-1}. The reaction was monitored for 5 min at 405 nm. Velocities of reaction were calculated. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer instead of inhibitor (extract, essential oil or decoction). Inhibitory activity was calculated from 100 subtracted by the percentage of enzyme activity. Data presented here are the average of three replicates.

The essential oils and the ethanolic extracts were redissolved in the minimum quantity of DMSO or ethanol, respectively. Decoctions were not evaporated and they were used as obtained. The quantities used in the cuvettes were equivalent to 0.5 mg and 1 mg of essential oil or ethanolic extract/ml. Decoctions were diluted to obtain three different concentrations equivalent of 0.5, 1 and 5 mg of dried plant material.

2.5. Antioxidant activity

DPPH assay: the method described by Tepe et al. (2005) was used. To 5 ml of a methanolic solution of 2,2′-diphenylpicrylhydrazyl (DPPH) 0.002% in methanol, 50 μl extract were added and the mixture was left at room temperature for 30 min. The absorption was measured at 517 nm against a corresponding blank.

β-Carotene-linoleic acid assay: the method described by Tepe et al. (2005) was used with a slight modification. A stock solution of β-carotene and linoleic acid was prepared by dissolving 0.5 mg of β-carotene in 1 ml of chloroform and adding 25 μl of linoleic acid together with 200 mg of Tween 40. The chloroform was evaporated. One hundred ml of aerated water were added to the residue. To 2.5 ml of this mixture 300 μl of each extract were added. The test tubes were incubated in boiling water for 2 h together with two blanks, one containing the antioxidant BHT and the other one without antioxidant. With this latter tube a complete oxidation is obtained with the initial yellow colour vanishing, while in the test tube with BHT the yellow colour will be maintained during the incubation period. The absorbance was measured at 470 nm.

Table 4
Effect on AChE activity, % of inhibition, of the decoctions ($n = 3$)

Plan	Decoction (mg plant/ml of assay)		
	0.5 mg ml^{-1}	1 mg ml^{-1}	5 mg ml^{-1}
Hypericum undulatum	12.6 ± 0.7	59.8 ± 0.9	81.7 ± 3.4
Laurus nobilis	19.9 ± 3.9	36.2 ± 2.4	56.1 ± 5.5
Lavandula angustifolia	n.i.	n.i.	n.i.
Lavandula pedunculata	n.i.	n.d.	67.8 ± 10.7
Malva silvestris	n.i.	n.i.	25 ± 5.7
Melissa officinalis	n.i.	12.8 ± 1.2	53.1 ± 2.2
Mentha suaveolens	n.i.	49.5 ± 5.1	68.9 ± 2.5[a]
Paronychia argentea	n.i.	7.8 ± 3.4	26.1 ± 1.2
Salvia officinalis	n.i.	6.0 ± 8.1	57.2 ± 15.9
Sanguisorba minor	n.i.	7.1 ± 1.6	n.d.

n.i.: no inhibition (inhibition ≤ 5%), n.d.-not determined.
[a] Concentration of 2.5 mg dry plant ml^{-1}.

3. Results and discussion

3.1. AChE activity

Results for the essential oils and ethanolic extracts are shown in Table 3, and results for the decoctions are presented in Table 4. Most of the plant materials analyzed showed some inhibitory activity towards acetylcholinesterase.

3.1.1. Essential oils

Essential oils exhibited moderate inhibitory activity (low: 5–25%; moderate: 25–50%; good: 50–100%) at a concentration of 0.5 mg ml^{-1}. The poorest results were obtained with the essential oils of *Melissa officinalis*, *Hypericum undulatum* and *Lavandula angustifolia*. Due to precipitation or turbidity in the test conditions, activity of the essential oil of *Malva silvestris* could only be determined at a concentration of 0.1 mg ml^{-1} and essential oils of *Laurus nobilis* and *Salvia officinalis* could not be tested at concentrations of 1 mg ml^{-1}.

Melissa officinalis and *Salvia officinalis* are reputed in old European books on medicinal herbs as having memory improving properties (Perry et al., 1999). Recent studies have

Table 3
Effect on AChE activity, % of inhibition, of the essential oils and ethanolic extracts ($n = 3$)

Plant, Scientific name	Essential oil		Ethanolic extract	
	0.5 mg ml^{-1}	1 mg ml^{-1}	0.5 mg ml^{-1}	1 mg ml^{-1}
Hypericum undulatum	20.0 ± 6.5	30.3 ± 19.7	68.4 ± 4.7	n.d.
Laurus nobilis	51.3 ± 1.7	n.d.	48.4 ± 6.9	64.3 ± 9.0
Lavandula angustifolia	33.7 ± 7.2	39.5 ± 8.6	26.6 ± 9.5	28.4 ± 3.8
Lavandula pedunculata	56.5 ± 4.9	48.3 ± 3.1	16.7 ± 8.6	42.0 ± 16.8
Malva silvestris	28.1 ± 2.9[a]	n.d.	n.i.	n.i.
Melissa officinalis	6.5 ± 9.2	65.3 ± 4.9	17.8 ± 6.9	n.d.
Mentha suaveolens	46.2 ± 10.3	52.4 ± 2.5	19.3 ± 3.9	27.1 ± 2.7
Paronychia argentea	44.6 ± 1.8	49.5 ± 1.0	48.7 ± 6.1	n.d.
Salvia officinalis	46.4 ± 11.9	n.d.	16.4 ± 5.4	n.d.
Sanguisorba minor	38.8 ± 7.3	46.1 ± 9.7	57.1 ± 9.1	77.5 ± 2.2

n.d.: not determined., n.i.: no inhibition (inhibition ≤ 5%).
[a] Concentration of 0.1 mg ml^{-1}.

shown that *Melissa officinalis* ethanolic extracts (Wake et al., 2000) and the dried plant (Kennedy et al., 2003), had in vitro cholinergic receptor binding properties, improved memory performance and increased calmness in 20 healthy young volunteers, with a dose of 1600 mg of encapsulated dried leaf (Kennedy et al., 2003). We found that the essential oil of *Melissa officinalis* under study inhibited acetylcholinesterase in a dose dependent way (0.5 mg ml^{-1}, 6.5 ± 2%; 0.6 mg ml^{-1}, 9.5 ± 5.3%; 0.7 mg ml^{-1}, 22.9 ± 2.7%; 0.8 mg ml^{-1}, 31.5 ± 7.1%; 1 mg ml^{-1}, 65.3 ± 4.9%). The 65% inhibition was obtained with an amount of essential oil equivalent to 1013 mg of plant (Table 2). Results obtained by the cited authors (Kennedy et al., 2003) may be due, in part, to the fact that they used the powdered plant and the present work used concentrated oil extract.

The inhibitory properties of *Salvia officinalis* are well documented in literature (Wake et al., 2000; Akhondzadeh et al., 2003; Savelev et al., 2004). Essential oil of *Salvia officinalis* inhibited 46% of acetylcholinesterase activity at a concentration of 0.5 mg ml^{-1}. It was not possible to test higher concentrations due to appearance of turbidity in test conditions.

Essential oil of *Lavandula angustifolia* is used in aromatherapy due to its calming and relaxing properties. *L. stoechas* is used for headache. It was believed in the XVI century that *Lavandula angustifolia* also could enhance intelligence (Cananagh and Wilkinson, 2002). Essential oils of *L. angustifolia* and *L. pedunculata* exhibited moderate inhibitory properties, 39.5% and 48.3%, respectively, for the concentration of 1 mg ml^{-1}. Essential oil of *Mentha suaveolens* also exhibited moderate inhibitory activity, 49.5% for the same concentration.

3.1.2. Ethanolic extract

Ethanolic extracts were generally less potent than essential oils, except for *Melissa officinalis*, *Sanguisorba minor* and *Hypericum undulatum*. Determination of inhibition by test solutions containing 1 mg ml^{-1} of extracts obtained from *Melissa officinalis*, *Hypericum undulatum* and *Salvia officinalis* was not possible due to their intense green colour. The best inhibitory results were obtained with the ethanolic extract of *Hypericum undulatum*, a 68.4% of inhibition. Ethanolic extract of *Sanguisorba minor* also have good inhibitory properties and exhibits a dose dependent relationship (0.3 mg ml^{-1}, 35.0 ± 6.1%; 0.5 mg ml^{-1}, 57.1 ± 9.1%; 0.7 mg ml^{-1}, 68.5 ± 6.1%; 1 mg ml^{-1}, 77.5 ± 2.2%) that also suggests a possible therapeutic value.

3.1.3. Decoctions

Decoctions were obtained boiling small pieces of dried plant in water for 20 min. This is much longer than recommended by herbalists but, for plants used as condiments, more similar to the recipes used in food preparation. Most of the solutions exhibited some inhibitory activity (Table 4) except *Lavandula angustifolia* where no inhibition could be detected even at higher concentrations. The more active diluted solutions (0.5 mg ml^{-1}) were those obtained from *Laurus nobilis* and *Hypericum undulatum* that had an inhibitory activity of 20% and 13%, respectively. A value of 56% and 82% is obtained when using extract corresponding to 5 mg of each plant, respectively. Laurel is a very common condiment in Portuguese cuisine and is used in preparation of meat, stews, rice, *pasta*, fish and even soups, as a part of everyday cuisine. It is interesting that the use of two small leaves (about 500 mg) in half a litre of liquid is quite common in many recipes. Decoctions of *Mentha suaveolens*, also used as condiment in soups, had an intense dark colour when testing solutions with concentrations higher than 2.5 mg of dried plant. Inhibitory values at this concentration are quite good, 68.9%. In general, more activity was obtained when using more concentrated decoctions of all the plants studied. Decoctions of *Paronychia argentea* and *Malva silvestris* however had poor inhibitory activity. The activity of *Sanguisorba minor* was not determined due to the intense dark colour of the decoction. The best results (81% inhibition) were obtained with *Hypericum undulatum*, and a decoction prepared from an amount of plant material similar to, or even less, than the one used in folk tradition. A related species, *Hypericum perforatum*, is one of the most useful natural therapeutic agents in the treatment of moderate and mild depression. Recent studies carried out with the loose pact clamp in the mouse neuromuscular junction indicated a potentiation of the acetylcholine action at the mouse neuromuscular junction. The authors suggested that *Hypericum perforatum* could have a possible action on acetylcholinesterase in terms of reduction of the degradation rate of acetylcholine (Re et al., 2003). The results presented here indicate that *Hypericum undulatum* may have the same effect and its use is of therapeutic value.

3.2. Antioxidant activity

The antioxidant activity of essential oils, ethanolic extracts and decoctions was evaluated by two tests: DPPH and β-carotene-linoleic acid extracts. As no relationship between the concentration of extracts and the antioxidant effect was evaluated, the results for all three extracts are presented in Table 5. The DPPH test intends to measure the hydrogen atom or electron donor capacity of the extracts to the stable radical DPPH formed in solution (Tepe et al., 2005). It measures the capacity of the extract to scavenge free radicals in solution. The antioxidant activity measured as the inhibition of oxidation of linoleic acid can simulate the oxidation of the membrane lipid components and also measures the capacity of inhibition of conjugated diene hydroperoxide arising from the linoleic acid oxidation (Tepe et al., 2005). This test measures the antioxidant activity toward linoleic acid relatively to β-carotene. This one is not affected due to the presence of a strong antioxidant. In this case the solution shall remain with the same initial colour meaning that β-carotene was not necessary to prevent the oxidation of linoleic acid as the antioxidant present in the extract was able to do so.

In most cases the highest radical scavenger capacity is found in the ethanolic extract, but the decoctions showed the higher values for the antioxidant activity measured with β-carotene-linoleic acid, Table 5. The β-carotene assay could not be performed with the ethanolic extract due to a strong green colour and the precipitate formed with all the plant extracts.

Table 5
Antioxidant activity (%) of essential oils and ethanolic extracts (0.1 mg ml^{-1}) and decoctions (0.1 mg dry plant ml^{-1} water)

Plant	Essential oil		Ethanolic extract		Decoction	
	DPPH	β-Carotene	DPPH	β-Carotene	DPPH	β-Carotene
Hypericum undulatum	57	64	85	n.d.	96	92
Laurus nobilis	53	44	67	n.d.	61	51
Lavandula angustifolia	n.a.	n.a.	23	n.d.	94	10
Lavandula pedunculata	10	33	70	n.d.	93	20
Malva silvestris	n.d	77[a]	n.a.	n.d.	30	87
Melissa officinalis	8	52	76	n.d.	96	46
Mentha suaveolens	n.a.	32	98	n.d.	94	65
Paronychia argentea	13	28	8	n.d.	9	52
Salvia officinalis	7	33	69	n.d.	80	84
Sanguisorba minor	11	99	93	n.d.	93	95

n.d.-not determined, n.a.- no activity (activity ≤ 5%).
[a] 0.03 mg dry plant ml^{-1} water.

Previous work carried out with *Salvia tomentosa* and other *Salvia* species (Tepe et al., 2005), *Phellinus baumii* (Shon et al., 2003), *Pimpinella anisum* (Gulçin et al., 2003), and various species from France (Trouillas et al., 2003) and Italy (Sacchetti et al., 2005) also obtained higher antioxidant activity in polar plant extracts. This suggests that polyphenols, flavanones and flavonoids known to have antioxidant activity (Cao et al., 1997; Lien et al., 1999) may be responsible (Tepe et al., 2005).

Sanguisorba minor showed antioxidant activity in all the fractions, essential oil, ethanolic and water extracts. The recently described phenolic compounds present in *S. minor* (Ayoub, 2003) may be responsible for the high antioxidant activity.

Melissa officinalis, *Mentha suaveolens*, *Salvia officinalis*, *Lavandula angustifolia* and *Lavandula pedunculata* showed appreciable antioxidant activity only in the polar fractions. *Laurus nobilis*, *Sanguisorba minor* and *Hypericum undulatum* showed antioxidant activity in all three extracts. Antioxidant activity of an ethanolic extract of *Hypericum perforatum* has previously been reported by Silva et al. (2004, 2005). They found that flavonoids aglycones were responsible for the free radical scavenging activity, and that induced lipid peroxidation in rat cultured hippocampal neurons was significantly inhibited by fractions containing flavonol glycosides, flavonol and biflavone aglycones or chlorogenic acid type phenolics present in the ethanolic extract. To our knowledge no previous studies on both radical scavenger and β-carotene-linoleic acid test had been reported for the essential oils and infusions or decoctions of *H. undulatum*.

In laurel (*Laurus nobilis*) isoquercitrin (Kang et al., 2002) and flavonol glycosides (Fiorini et al., 1998) may account for the antioxidant activity exhibited.

4. Conclusions

Among the 10 Portuguese plants analyzed several showed inhibitory activity of the enzyme acetylcholinesterase and antioxidant activity. *Hypericum undulatum*, *Melissa officinalis*, *Laurus nobilis* and *Lavandula pedunculata* showed high values for both. The ethanolic extract of *Sanguisorba minor* showed the best inhibition of AChE and a very good antioxidant activity. A decoction of *Mentha suaveolens* was also very effective in the inhibition of AChE and as a scavenger of radicals. *Laurus nobilis*, *Mentha suaveolens*, used as condiments or as medicinal plants, and *Hypericum undulatum, Melissa officinalis and Sanguisorba minor*, used as medicinal plants, may help in preventing or alleviating patients suffering from AD as they showed both inhibitory activity of AChE and antioxidant activity.

Finally, it is interesting to note that herbs that have been used for a long time as food, condiments and medicine in Portugal have, in fact, properties that may suggest new applications.

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