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Acetylcholinesterase inhibition and antioxidant activity of the water extracts of several *Hypericum* species

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ABSTRACT

Many *Hypericum* species are widely used in Portugal, as well as in many other countries. In order to define the main components present in the water extracts, three *Hypericum* species, *H. androsaemum* and *H. perforatum*, obtained in the local supermarket, and *H. undulatum*, collected in an interior region of Portugal, were analysed for their inhibition of acetylcholinesterase and antioxidant activity. *H. perforatum* from five different suppliers was tested. All species were active, with IC_{50} values between 0.62 ± 0.06 and 1.79 ± 0.37 mg dry extract/ml. HPLC–diode array analysis indicated that chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin were the main compounds present in the water extracts. Their inhibitory activities, determined as IC_{50} values, were 196, 86, 66, 63 and 62 μg/ml, respectively. The highest inhibitory activity was found with *H. androsaemum* and *H. undulatum*. All the species showed high antioxidant activity ($DPPH^{\cdot}$ test). Values of EC_{50} between 9.0 ± 0.2 and 18.4 ± 0.8 μg of dry extract/ml were obtained.

1. Introduction

Hypericum perforatum, also known as St. John's wort, has been used in the treatment of mild depression (Eggelkraut-Gottanka, Abed, Muller, & Schmidt, 2002; Kasper et al., 2008; Pilkington, Boshnakova, & Richardson, 2006; Wurglics & Schubert-Zsilavecz, 2006) all over the world. Portugal is no exception (Cunha, Silva, & Roque, 2003). Several studies refer to hypericin (Eggelkraut-Gottanka et al., 2002; Sauviat, Colas, Chauveau, Drapier, & Negrerie, 2007) and hyperforin (Eggelkraut-Gottanka et al., 2002; Leuner et al., 2007; Menegazzi et al., 2008; Zhou et al., 2004) as the compounds in *Hypericum perforatum* that may explain its biological activity. Most of the studies carried out with this plant refer to polar organic extracts (Brolis et al., 1998; Gioti, Skalkos, Fiamegos, & Stalikas, 2005; Liu, Pan, Drumm, & Ang, 2005; Silva, Malva, & Dias, 2008; Tatsis et al., 2007; Williams, Sander, Wise, & Girard, 2006). Very few studies deal with the water extracts, e.g. infusions or decoctions.

Depression is also a condition found in people suffering from Alzheimer disease (AD) and one of the processes to alleviate the symptoms is to inhibit acetylcholinesterase (AChE) (Heinrich & Teoh, 2004), the enzyme that catalyses the hydrolysis of the neurotransmitter, acetylcholine. This way to tackle the problem involves the use of drugs that have been the objective of much research. One of the active compounds in use, for instance galantamine, was isolated from the plant genus *Galanthus* (Latvala et al., 1995). If *H. perforatum* could show this double action, alleviate depression and simultaneously inhibit AChE, it might be interesting for AD treatment.

The action of *Hypericum*, on the central nervous system, was previously attributed to the presence hypericin and hyperforin (Verotta, 2003). Although the amounts of hypericin and hyperforin depend on the plant analysed, and also on the extraction procedure, they are usually not the main components of *H. perforatum*. They are found in smaller concentrations than the flavonoids rutin, hyperoside, isoquercitrin, quercetin and the caffeic acid derivative, cholorogenic acid (Brolis et al., 1998; Williams et al., 2006). These molecules may also participate in the biological activity of *H. perforatum* (Paulke, Noldner, Scubert-Zsilavecz, & Wurglics, 2008).

The antioxidant activity is also relevant in the treatment of Alzheimer's disease (Frank & Gupta, 2005; Resende et al., 2008). It was demonstrated that the absence of the natural antioxidant, vitamin E, enhanced AD in a mouse model (Nishida et al., 2006). The endogenous formation of free radicals can contribute to the inflammatory processes (Gomes, Fernandes, Lima, Mira, & Corvo, 2008). It is known that inflammation can also contribute to the development of the disease (Cunningham et al., 2008; Teeling & Perry, 2008).

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With the objective of finding whether the *Hypericum* species, commercially available, drunk as a hot beverage, could contribute to the inhibition of acetylcholinesterase, water extracts of this plant were analysed for their AChE inhibition and antioxidant activity. *H. androsaemum and H. perforatum*, from different suppliers, and *H. undulatum*, collected in the east of Portugal, Beira Baixa, were the targets of this study. The main compounds responsible for the enzyme inhibition were determined by HPLC–DAD, comparatively to standards.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Acetylcholinesterase (AChE) type VI-S, from electric eel, 349 U/mg of solid, 411 U/mg of protein, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), acetylthiocholine iodide (AChI), tris[hydroxymethyl]aminomethane (tris buffer), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,6-di-tert-butyl-4-hydroxytoluene (BHT), pyrogallol, chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin, quercetin and Folin–Ciocalteau reagent were obtained from Sigma.

2.2. Plant material

H. androsaemum and *H. perforatum* were obtained from a local supermarket; materials from five different suppliers were analysed (Table 1).*H. undulatum* was collected in the east region of the country, Beira Baixa. A voucher specimen (LISUL204117) was deposited in the herbarium of the Faculty of Science, University of Lisbon.

2.3. Extract preparation

Aqueous plant extracts were prepared as decoctions by using 10 g of ground dry plant material in 100 ml of distilled water, boiling for 10 min. The decoctions were filtered through Whatman paper. Aliquots of 1 ml of each extract were lyophilised and used to determine the dried weight. Data, presented in Table 1, are the averages of three independent measures.

2.4. Acetylcholinesterase inhibition

Acetylcholinesterase enzymatic activity was measured as described in Falé et al. (2009); briefly, 90 μl of 50 mM Tris–HCl buffer, pH = 8, 30 μl of sample and 7.5 μl of acetylcholinesterase solution containing 0.26 U/ml were mixed in a microwell plate and left to incubate for 15 min. Subsequently, 22.5 μl of a solution of AChI (0.023 mg/ml) and 142 μl of 3 mM DTNB were added. The absorbance was read at 405 nm when the reaction reached equilibrium. A control reaction was carried out using water instead of extract and it was considered 100% activity.

$$I(\%) = 100 - (A_{\text{sample}}/A_{\text{control}}) \times 100$$

where A_{sample} is the absorbance of the extract containing reaction mixture and A_{control} the absorbance of the control reaction. I is % inhibition. Tests were carried out in triplicate and a blank with Tris–HCl buffer instead of enzyme solution was used. In the case of the standards, a blank with methanol was carried out as these compounds were dissolved in this organic solvent.

2.5. Determination of antioxidant activity

Antioxidant activity was measured by the DPPH$^{\cdot}$ method, as described in Falé et al. (2009); briefly, to 5 ml of a solution of DPPH$^{\cdot}$ 0.002% in methanol, 50 μl of plant extract were added. The mixture was incubated for 30 min at room temperature. The absorbance was measured at 517 nm against a corresponding blank. The antioxidant activity (AA) was calculated as:

$$\text{AA}(\%) = 100 \times (A_{\text{DPPH}} - A_{\text{sample}})/A_{DPPH}$$

where A_{DPPH} is the absorption of the DPPH$^{\cdot}$ solution against the blank, and A_{sample} is the absorption of the extract against the blank. The test was carried out in triplicate and the extract concentration providing 50% of antioxidant activity (EC_{50}) was obtained by plotting the antioxidant activity against the plant extract concentration.

2.6. Determination of total phenolic compounds

Total phenolic compound content was determined spectrophotometrically, as described by Falé et al. (2009), using pyrogallol as a standard; briefly, 100 μl of the extract solution containing 1 mg of the extract/ml were diluted with 4.5 ml of water. To this solution, 100 μl of Folin–Ciocalteu were added. After 3 min, 300 μl of a 2% solution of sodium carbonate were added and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance (A) was measured at 760 nm and a calibration curve was established:

$$A = 1.437\text{pyrogallol} + 0.0065$$

The concentration of the total phenolic compounds was determined as microgrammes of pyrogallol equivalents by using the equation above as the mean of three replicates (mg of pyrogallol equivalents per mg of dry extract).

2.7. HPLC analysis

The HPLC analysis was carried out in a Liquid Chromatograph Finnigan TM Surveyor Plus Modular LC System equipped with a diode array detector and a Lychromasphere 5 μm C_{18} column from Merck, and Xcalibur software. The extracts were filtered through 0.2 μm filters and analysed by HPLC, injecting 25 μl and using a gradient composed of solution A (acetonitrile), solution B (water with 0.05% trifluoracetic acid) and solution C (methanol) as follows: t = 0, eluent A: 10%, eluent B: 90%, eluent C: 0%; t = 50 min, eluent A: 80%, eluent B: 5%, eluent C: 15% methanol. The standards were run under the same conditions, using 0.1 mg/ml solutions in methanol and the detection was carried out between 200 and 600 nm. The chromatograms were obtained at photo diode array (PDA) values.

Table 1
Source and abbreviations of the *Hypericum* species used in the present work as well as the mass of dry extract obtained (mg/mg).

Plant	Source	Designation	mg Dry extract/mg plant
H. androsaemum	Local supermarket-supplier A	HAd	0.299
H. undulatum	Herbarium no. LISU 204117	HUn	0.391
H. perforatum	Local supermarket-supplier A	HP_1	0.292
H. perforatum	Local supermarket-supplier B	HP_2	0.368
H. perforatum	Local supermarket-supplier C	HP_3	0.263
H. perforatum	Local supermarket-supplier D	HP_4	0.297
H. perforatum	Local supermarket-supplier E	HP_5	0.201