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In vitro multimodal-effect of *Trichilia catigua* A. Juss. (Meliaceae) bark aqueous extract in CNS targets

João Bernardo[a], Federico Ferreres[b,*], Ángel Gil-Izquierdo[b], Romeu António Videira[a], Patrícia Valentão[a], Francisco Veiga[c,d], Paula B. Andrade[a,*]

[a] REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, no. 228, 4050-313 Porto, Portugal

[b] Research Group on quality, Safety and Bioactivity of Plant Foods, Department of Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus University Espinardo, Murcia, Spain

[c] REQUIMTE/LAQV, Group of Pharmaceutical Technology, Faculty of Pharmacy, Universidade de Coimbra, Coimbra, Portugal

[d] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra (FFUC), Pólo das Ciências da Saúde, Azinhaga de Santa Comba 3000-548 Coimbra, Portugal

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ABSTRACT

Ethnopharmacological relevance: The bark of *Trichilia catigua* A. Juss. (Meliaceae), popularly known as "big catuaba", is traditionally used in Brazilian folk medicine for its neuroactive potential as memory stimulant, and antinociceptive and antidepressant effects.

Aim of the study: To study the aqueous extract of *T. catigua* bark as dual inhibitor of monoamine oxidase A (MAO-A) and acetylcholinesterase (AChE). To explore its antioxidant potential through interaction with xanthine/xanthine oxidase (X/XO) pathway, and to attempt a relationship between its phenolic profile and effects displayed.

Materials and methods: Phenolic profiling was achieved by HPLC-DAD-ESI/MS^n and UPLC-ESI-QTOF-MS analyses. The capacity to inhibit *h*MAO-A was assessed *in vitro*, as was that for AChE, evaluated in rat brain homogenates. The direct inhibition of the X/XO pathway and the scavenging of superoxide anion radical were the selected *in vitro* models to explore the antioxidant potential. The cytotoxic effects were assayed in the human neuronal SH-SY5Y cells by MTT reduction, after direct exposure (24 h).

Results: Twenty-six compounds were identified and quantified (551.02 ± 37.61 mg/g of lyophilized extract). The phenylpropanoid substituted flavan-3-ols were the most representative compounds (~81% of quantified mass). The extract inhibited *h*MAO activity in a concentration-dependent manner (IC_{50} = 121.06 ± 2.13 μg/mL). A mixed model of inhibition of AChE activity was observed, reflected by the pronounced increase of *K*m values and a more discreet effect over the *V*max parameters, calculated from Michaelis-Menten fitted equations. In addition, it was demonstrated that the extract directly inhibits the X/XO pathway (IC_{50} = 121.06 ± 2.13 μg/mL) and also imbalances the oxidative stress acting as superoxide anion radical scavenger (EC_{50} = 104.42 ± 10.67 μg/mL), an oxidative by-product of this reaction. All these neuroprotective and neurotrophic effects were displayed within the non-toxic range of concentrations (0.063–0.500 μg/mL) in SH-SY5Y cells.

Conclusions: Our results validate the traditional use of *T. catigua* bark for its neuroactive and neuroprotective potential. A novel approach upon its application towards the management of neurodegenerative and related symptomatology was likewise demonstrated.

1. Introduction

Medicinal species are a continuing source of new bioactive compounds, and simultaneously offer a diversified platform of scaffolds to design novel drug leads (Cragg and Newman, 2013). Ethnopharmacological oriented studies are a sustained and common perspective to search for bioactive natural products, and bridge the connection between popular knowledge and positive health outcomes (Helmstädter and Staiger, 2014).

The decoctions of *Trichilia catigua* A. Juss. bark (Meliaceae), a Brazilian native species popularly known as "big catuaba", are traditionally used in Brazilian folk medicine for its neuroactive potential as

* Corresponding authors.
E-mail addresses: federico@cebas.csic.es (F. Ferreres), pandrade@ff.up.pt (P.B. Andrade).

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memory stimulant, and antinociceptive and antidepressant effects (Dutra et al., 2016; Longhini et al., 2016). The medicinal applications of this species are consistent with some published studies that focused its neuroactive potential. In mouse nociception behavioural models, the hydroalcoholic extract (200 mg/kg, *p.o.*) displayed antinociceptive effects associated with the activation of the dopaminergic system and, to a lesser extent, through interaction with opioid pain pathway (Calixto et al., 2011). A dopamine-mediated antidepressant-like effect was observed for mice after acute oral treatment with *T. diffusa* bark hydroalcoholic extract (200 mg/kg *p.o.*, 6 h), sustained by the *in vitro* uptake inhibition and increased release of serotonin, and especially of dopamine, in rat brain synaptosomal preparations (Campos et al., 2005). It was also demonstrated that polyphenolic rich extracts of this species exert valuable antioxidant, anxiolytic and antidepressant activities, which validates its possible application as supporting treatment for mood disorders (Chassot et al., 2011; Taciany Bonassoli et al., 2012).

T. catigua bark alcoholic and hydroalcoholic extracts, traditionally designated as “Catuama”, are characterized as a complex mixture of polyphenols, such as phenolic acids, flavonoids, flavonolignans and other related compounds (Longhini et al., 2016). Notwithstanding the issue of their bioavailability (Bernardo et al., 2016), these natural compounds are multi-target ligands, which interact with Central Nervous System (CNS) elements and prevent or delay the progression of Major Depressive Disorder (MDD) or of the neurodegenerative Alzheimer's (AD) and Parkinson's diseases (PD) (Grosso et al., 2013). Considering that neurological affections have a complex aetiology, there is an encouragement to develop multi-functional therapeutic strategies with complementary biological activities, in order to provide greater symptomatic efficacy and to increase patients’ health gains (Liu et al., 2016; Yáñesz and Viña, 2013). Some proposed examples to manage cognition impairment, motor dysfunction, MDD and neurodegeneration combine the inhibition of monoamine oxidase (MAO) and acetylcholinesterase (AChE) with anti-inflammatory and antioxidant activities, and also embrace other neuroprotective mediated events (Youdim and Buccafusco, 2005).

Our main goal was to explore the potential of *T. catigua* bark aqueous extract to modulate/interact with other CNS targets underpinning neurodegeneration. We studied its multimodal-effect as dual inhibitor of MAO-A and AChE, its neurotrophic potential to impair xanthine oxidase (XO) mediated oxidative stress and effects over the viability of the human neuronal cell model line SH-SY5Y. In contrast with the majority of published reports, we studied the chemical profile of an aqueous extract instead of ethanolic preparations. A relationship between its phenolic profile and the displayed activities will be attempted, which may represent an important advance to the understanding of the traditional use of this medicinal species, and open new perspectives and applications within the fields of ethnopharmacology and medicinal chemistry.

2. Materials and methods

2.1. Plant material

Trichilia catigua A. Juss. bark (lot 3553.204.13) was acquired to Morais e Costa & CA. Lda (Porto, Portugal). The voucher specimen was deposited at Laboratory of Pharmacognosy, Faculty of Pharmacy of University of Porto (TC-B-112016). The authenticity of the biological material was ensured by the supplier's technical sheet and further macroscopic and microscopic analyses performed by the authors. The plant material was stored in desiccators, in the dark, at room temperature, to avoid deterioration and physicochemical alterations.

2.2. Preparation of the aqueous extract

The plant material was powdered, sieved (≤ 910 μm) and the aqueous extract was prepared by decoction (*ca.* 3 g in 500 mL of water, for 20 min at 100 ± 5 °C). After filtration, under reduced pressure, the extract was lyophilized and stored.

2.3. Standards and reagents

Catechin, epicatechin, and epigallocatechin were purchased at Extrasynthese (Genay, France). 3-Caffeoylquinic acid, 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, acetonitrile, acetylthiocholine iodide, clorgyline, dimethyl sulfoxide (DMSO), ethylenedinitrilotetraacetic acid (EDTA), ethopromazine, formic acid, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), human monoamine oxidase-A (*h*MAO-A) (EC 1.4.3.4), methanol, methylthiazolyldiphenyl-tetrazolium bromide (MTT), ß-nicotinamide adenine dinucleotide reduced form (NADH), nitroblue tetrazolium chloride (NBT), phenazine methosulfate (PMS), sodium hydroxide, sucrose, xanthine (X), xanthine oxidase (XO) (EC 1.1.3.22) and kynuramine were acquired from Sigma-Aldrich (St. Louis, MO, USA). 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased to Alfa Aesar (Karlsruhe, Germany). Dulbecco's Modified Eagle Medium / Nutrient Mixture F-12 (DMEM/F12), fetal bovine serum (FBS), Pen-Strep solution and trypsin-EDTA (0.05%) were obtained from Gibco (Invitrogen, Paisley, UK). The SH-SY5Y cell line (ATCC® CRL-2266™) was acquired to American Type Culture Collection (Manassas, Virginia, USA).

2.4. Phenolic profiling of T. catigua bark lyophilized aqueous extract

2.4.1. HPLC–DAD–ESI/MS^n qualitative analyses

The chromatographic analysis was performed as described before (Ferreres et al., 2015a, 2015b), with a minor alteration of the gradient: the mobile phase consisted of acidified water (1% formic acid; A) and acetonitrile (B), and the elution started with 5% B to obtain 30% B at 30 min, and 50% B at 40 min. Data was recorded at 260, 280, 330 and 530 nm, and full scan mass covered the range from *m/z* 100 up to 1500.

2.4.2. UPLC-ESI-QTOF-MS qualitative analysis

The determinations of exact mass were performed with an Agilent 1290 Infinity LC system coupled to the 6550 Accurate-Mass QTOF (Agilent Technologies, Waldbronn, Germany) with an electrospray interface (Jet Stream Technology). The mobile phase combined acidified water (0.1% formic acid; A) and acidified acetonitrile (0.1% formic acid; B), and compounds were separated using the following gradient conditions: start with 5% B to obtain 30% B at 12 min, and 50% B at 15 min. The extract was reconstituted (1 μL) and was eluted in a Luna Omega reversed phase column (1.6 μm, PS C18, 100 Å, 50 × 2.1 mm; Phenomex, Macclesfield, UK), protected with SecurityGuard ULTRA Cartridges of the same material (flow rate of 0.5 mL/min, 30 °C). The optimal electrospray interface conditions were the following: gas temperature 280 °C, drying gas flow rate 11 L/min, nebulizer pressure 45 psi, sheath gas temperature 400 °C and sheath gas flow 12 L/min. The MS system was operated in negative ion mode with the mass ranging between *m/z* 50 and1500, in full scan resolution mode. The remaining conditions were described in a previous work (Garcia et al., 2016).

2.4.3. HPLC–DAD quantification of phenolic compounds

The analytical conditions were the same described above for compounds identification. Reconstituted lyophilized extract and standard solutions (20 μL) were injected on a HPLC-DAD unit (Gilson Medical Electronics, Villiers le Bel, France) after filtration (0.45 μm). Detection was achieved with an Agilent 1100 series DAD (Agilent Technologies, Waldbronn, Germany), and chromatograms were recorded at 280 and 320 nm (Clarity Software version 5.04.158, DataApex Ltd, Prague, Czech Republic). Compounds were quantified by interpolation with external standard curves (Table 1), as follows: 3- and 4-quinic acid esters (**1**–**3** and **6**) were quantified as 3-caffeoylquinic acid; 5-quinic acid esters (**5** and **7**) as 5-caffeyoilquinic acid; compound **25** was