SUPPLEMENTARY MATERIAL

Antiedematogenic activity, acetylcholinesterase inhibition and antimicrobial properties of *Jacaranda oxyphylla*

V.V. Pereira^{ab*}, R.R. Silva^c, M.H. dos Santos^d, D.F. Dias^e, M.E.C. Moreira^e, J.A. Takahashi^a

^aDepartamento de Química, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, Brazil;

^bFaculdade de Farmácia, Universidade Federal de Minas Gerais, 31270-901 Belo Horizonte, Brazil;

^cDepartamento de Química, Universidade Federal dos Vales do Jequitinhonha e Mucuri, 39100-000 Diamantina, Brazil; ^dDepartamento de Química, Universidade Federal de Viçosa, 36570-900 Viçosa, Brazil;

^eLaboratório de Fitoquímica e Química Medicinal, Universidade Federal de Alfenas, 37130-000, Alfenas, Brazil.

* Corresponding author. Email: vnscviana@yahoo.com.br

Jacaranda oxyphylla Cham. (Bignoniaceae) is a shrub found in the Brazilian cerrado and used in folk medicine to treat microbial infections. The aim of this study was to carry on a phytochemical screening and evaluate antiedematogenic, antimicrobial and antiacetylcholinesterase properties of *J. oxyphylla* crude extracts. All extracts analyzed showed presence of terpenoids, which are chemical substances potentially active. It was found a high AChE inhibitory activity for hexane extract from leaves and for the extracts from twigs. Ethanol extract from leaves of *J. oxyphylla* showed activity against Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Escherichia coli*) bacteria. This extract was also effective in inhibiting the stages of inflammation evaluated. Biological investigation and phytochemical screening of *J. oxyphylla* extracts provided additional evidence of its traditional medicinal value.

Keywords: Bignoniaceae, *Jacaranda oxyphylla*, anti-inflammatory, AChE inhibition, antibacterial activity.

Experimental

Plant material and extraction

The aerial parts of the plant were collected in São João da Chapada, near Diamantina city (MG, Brazil) in July 2012. The plant material was identified by Dr. L. H. Y. Kamino (Institute of Biological Sciences, Universidade Federal de Minas Gerais, Brazil). The voucher specimen of the plant material is deposited in the BHCb Herbarium of the Universidade Federal de Minas Gerais under registration n^o. 170.970.

Plant material was placed on the paper sheets and dried at room temperature until a constant weight. Leaves and twigs of *J. oxyphylla* were separated and powdered. Dried leaves (1.26 kg) and twigs (0.76 kg) were successively extracted with hexane, chloroform and ethanol by maceration, followed by filtration. The extracts were prepared at room temperature. Hexane, chloroform and ethanol extracts were concentrated under vacuum using a rotary evaporator (IKA, China) to afford crude hexane extract from leaves (HL= 13.0 g; 1.0%) and twigs (HT= 2.2 g; 0.3%), crude chloroform extract from leaves (CL= 56.4 g; 4.5%) and twigs (CT= 10.2 g; 1.3%) and crude ethanol extract from leaves (EL= 215.1 g; 17.1%) and twigs (ET= 49.3 g; 6.5%).

Phytochemical screening

Extracts from *J. oxyphylla* were subjected to phytochemical screening assay for the presence of terpenoids, saponins, flavonoids, tannins and alkaloids (Matos 1997; Sharapin 2000). Interpretation of results was based on the observation of color change in the reaction system.

Antiedematogenic assay

Extracts obtained from leaves and twigs of the *J. oxyphylla* species were submitted to the antiedematogenic bioassay. This assay was conducted at the Laboratory of Physiology, Universidade Federal de Alfenas (UNIFAL, MG, Brazil). To perform the anti-inflammatory assay (paw edema induced by carrageenan), crude extracts of leaves and twigs obtained from *J. oxyphylla* species were suspended in carboxymethylcellulose solution (CMC, 0.5% w V⁻¹). Indomethacin solubilized in Tris buffer and saline in 1:1 ratio was used as positive control and CMC as negative control (Wang et al. 2013).

The animals used were Swiss mice (25-35 g), which were obtained from the UNIFAL vivarium (UNIFAL Ethics Committee, protocol 488/2013). They were treated with commercial feed and water *ad libitum*, guaranteed its adaptation for 7 days at room temperature of 23 ± 2 °C with 12 h dark/light cycle in the appropriate polypropylene maintenance cycle boxes. They were deprived of food for 12 h prior to the experiment and at the end of the experiments the animals were sacrificed by halothane inhalation.

Evaluation of the anti-inflammatory activity via paw edema test was induced by injection of 40 µL of carrageenan (2% w V⁻¹) solubilized in sterile saline and administered into the subplantar region of the right hind paw of male mice (n = 8). One hour before injection of carrageenan, the animals were treated with the samples (oral via) at doses of 30, 100 and 300 mg kg⁻¹, or indomethacin (10 mg kg⁻¹), or CMC vehicle (10 mL kg⁻¹). The volume of the right paw of the animal was determined by immersing the tibio-tarsal region using a plethysmometer before carrageenan administration and one, two, three and four hours after receiving the carrageenan (Chavan et al. 2012).

Results were processed using GraphPad[®] software version 5.0 to determine mean ± standard error of mean (SEM). Analysis of variance met assumptions of the method followed by Newman-Keuls multiple comparisons test was conducted. Significant results were assumed for p < 0.05 in all calculations. To facilitate comparisons among the tested samples, the results were expressed as % of edema inhibition.

Acetylcholinesterase inhibition assay

Extracts obtained from leaves and twigs of the *J. oxyphylla* species were screened on a quantitative assay of the inhibitory activity of acetylcholinesterase (AChE), based on Ellman's method (1961).

The extracts (2.0 mg mL⁻¹) in dimethylsulfoxide (DMSO) were applied (25 µL) to 96 well microtiter plates, as well as (25 µL) of the aqueous solution of acetylthiocholine iodide (ACTI) 15 mM; (125 µL) of the solution of 5,5-dithiobis [2-nitrobenzoic acid] (DTNB) 3 mM and (50 µL) of Tris/HCl 50 mM pH 8, containing bovine serum albumin 0.1% (w V⁻¹). In parallel, a positive control was carried out, using eserine (2.0 mg mL⁻¹), and a negative control was prepared using the solvent DMSO (25 µL). The assay was performed in quintuplicate (Teles & Takahashi 2013).

Subsequently, eight readings were obtained (one every minute) on a microplate spectrophotometer (ELx800, BioTek, USA) at 405 nm (control). Once reading, solution of AChE (0.222 U mL⁻¹) in Tris/HCl 50 mM pH 8 containing bovine serum albumin 0.1% (w V⁻¹) were added (25 µL) to each well. The absorbance was measured again ten times. The results obtained were treated in Origin[®] version 6.0 program and expressed as % inhibition of mean ± standard deviation.

Antimicrobial screening

The extracts obtained from leaves and twigs of the *J. oxyphylla* species were subjected to antimicrobial screening by broth microdilution method with adaptations (Cueva et al. 2010). Two Gram-positive bacteria (*Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC 29212), two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella typhimurim* ATCC 14028) and the yeast *Candida albicans* ATCC 18804 were assayed. The microbial inocula were prepared with sterile distilled water until 74-75% of the absorbance in a spectrophotometer (SP-22 Biospectro, Brazil) at 625 nm.

The extracts were solubilized in DMSO to a concentration of 50 mg mL⁻¹ and were diluted in brain heart infusion (BHI) broth to achieve a concentration of 2.0 mg mL⁻¹. In 96-well microplates, 100 µL of the extracts were applied and the test samples were added to 100 µL of the microbial inocula previously prepared.

In parallel, it was prepared a blank assay (100 μ L of each sample diluted in BHI plus 100 μ L of sterile distilled water), the positive control (100 μ L of microbial strains plus 100 μ L of BHI broth) and negative control (100 μ L of sterile distilled water plus 100 μ L of BHI broth). Ampicillin was tested as antibacterial standard and nystatin as antifungal standard (2.0 mg mL⁻¹). Bioassay of each microorganism was performed on a different microplate and it was performed in quintuplicate.

The plates were incubated in an oven (Q-316, Quimis, Brazil) at 35 °C. After 24 and 48 h, absorbance (Abs) was reading on a microplate spectrophotometer (ELx800, BioTek, USA) at 495 nm. The results of this assay were expressed as % inhibition of antimicrobial growth.

References

- Chavan MJ, Wakte PS, Shinde DB. 2012. Analgesic and anti-inflammatory activities of the sesquiterpene fraction from *Annona reticulata* L. bark. *Nat. Pod. Res.* 26: 1515-1518.
- Cueva C, Moreno-Arribas MV, Martín-Alvare PJ, Bills G, Vicente MF, Basilio A, Rivas CL, Requena T, Rodríguez JM, Bartolomé B. 2010. Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. *Res. Microbiol.* 161: 372-82.
- Ellman GL, Courtney KD, Andres Jr V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* 7: 88-90.
- Matos FJA. 1997. Introduction to experimental phytochemical. Fortaleza: UFC.
- Sharapin N. 2000. Technology fundamentals of phytotherapeutic products. Santa Fé de Bogotá: Cyted.
- Teles AP, Takahashi JA. 2013. Paecilomide, a new acetylcholinesterase inhibitor from *Paecilomyces lilacinus*. *Microbiol Res.* 168: 204-210.
- Wang BS, Huang GJ, Lu YH, Chang LW. 2013. Anti-inflammatory effects of an aqueous extract of Welsh onion green leaves in mice. *Food Chem.* 138: 751-756.

Table S1 – Phytochemical analysis of the extracts from *J. oxyphylla*.

Extract ^a	Terpenoids	Saponins	Flavonoids	Tannins	Alkaloids
HL	+	–	–	–	–
CL	++	–	–	+	–
EL	++	++	++	+	–
HT	+	–	–	–	–
CT	++	–	–	+	–
ET	++	++	+	+	–

^aObtained from leaves (L) or twigs (T) with hexane (H), chloroform (C) or ethanol (E); ++ = strong presence, + = moderate presence, – = not detected.

Table S2 – *In vitro* antiacetylcholinesterase activity (iAChE) and growth inhibition of microorganisms induced by the extracts from *J. oxyphylla*.

Extract ^a or standard ^b	iAChE ^c	Microorganism growth inhibition ^c				
		<i>B. cereus</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>C. albicans</i>
		ATCC 1778	ATCC 29212	ATCC 25922	ATCC 14028	ATCC 18804
HL	77.6 ± 1.5	21.4 ± 2.4	0	77.4 ± 1.6	10.9 ± 1.4	29.5 ± 1.3
CL	40.3 ± 1.2	20.8 ± 0.6	39.5 ± 1.7	68.1 ± 1.3	31.6 ± 1.1	24.7 ± 1.2
EL	46.6 ± 1.1	96.9 ± 1.7	96.6 ± 2.0	15.2 ± 1.1	3.9 ± 0.8	25.3 ± 1.3
HT	84.1 ± 1.2	8.0 ± 1.5	29.0 ± 1.5	15.8 ± 1.5	14.5 ± 1.0	40.7 ± 1.3
CT	66.0 ± 1.0	61.6 ± 2.2	13.8 ± 1.9	96.3 ± 2.2	22.1 ± 0.9	39.7 ± 1.7
ET	71.5 ± 1.3	16.8 ± 1.7	22.2 ± 2.5	27.5 ± 1.3	2.1 ± 0.5	6.8 ± 0.8
Standard	97.2 ± 0.7	99.5 ± 0.4	99.2 ± 0.3	99.3 ± 0.2	99.1 ± 0.5	98.9 ± 0.9

^aObtained from leaves (L) or twigs (T) with hexane (H), chloroform (C) or ethanol (E); ^beserine for acetylcholinesterase assay, ampicillin for bacteria and nystatin for yeast; compounds were assayed in concentration of 2.0 mg mL⁻¹; ^cresults are mean values of quintuplicate assays ± standard deviation (expressed as % inhibition).

(A)

(B)

Figure S1 – Effects of the administration of the *J. oxyphylla* hexane extract from leaves - HL (Panel A) and hexane extract from twigs - HT (Panel B) at doses 30, 100 and 300 mg kg⁻¹, p.o.) or indomethacin (10 mg kg⁻¹, p.o.) on mice paw edema induced by subplantar carrageenan injection (1 mg per paw). Each point represents the mean $\pm$ S.E.M. of eight animals. The asterisks denote the significance levels when compared with the vehicle group: *** p<0.001, ** p<0.01., * p<0.05.

(A)

(B)

Figure S2 – Effects of the administration of the *J. oxyphylla* chloroform extract from leaves - CL (Panel A) and chloroform extract from twigs - CT (Panel B) at doses 30, 100 and 300 mg kg⁻¹, p.o.) or indomethacin (10 mg kg⁻¹, p.o.) on mice paw edema induced by subplantar carrageenan injection (1 mg per paw). Each point represents the mean $\pm$ S.E.M. of eight animals. The asterisks denote the significance levels when compared with the vehicle group: *** p<0.001, ** p<0.01., * p<0.05.

(A)

(B)

Figure S3 – Effects of the administration of the *J. oxyphylla* ethanol extract from leaves - EL (Panel A) and ethanol extract from twigs - ET (Panel B) at doses 30, 100 and 300 mg kg⁻¹, p.o.) or indomethacin (10 mg kg⁻¹, p.o.) on mice paw edema induced by subplantar carrageenan injection (1 mg per paw). Each point represents the mean $\pm$ S.E.M. of eight animals. The asterisks denote the significance levels when compared with the vehicle group: *** p<0.001, ** p<0.01., * p<0.05.