In vitro inhibition of acetylcholinesterase by crude plant extracts from Colombian flora

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The methanol extracts from five different plant families (Asteraceae, Euphorbiaceae, Melastomataceae, Rubiaceae, and Solanaceae) collected at Regional Natural Park Ucumarí (Colombia), were screened for their acetylcholinesterase inhibitory activity through the modified Ellman's spectrophotometric method. The best inhibitory activities on this study were shown by the extracts of Solanum leucocarpum *Dunal (IC_{50} = 204.59 mg/l) and* Witheringia coccoloboides *(Damm) (IC_{50} = 220.68 mg/l), both plants belonging to the Solanaceae family.*

Key words: acetylcholinesterase (AChE) inhibitors - Asteraceae - Euphorbiaceae - Melastomataceae - Rubiaceae - Solanaceae

The acetylcholinesterase enzyme (AChE) is an attractive target for the rational drug design and for the discovery of mechanism based inhibitors because of its role in the hydrolysis of the neurotransmitter acetylcholine (ACh). AChE inhibitors are the most effective approach to treat the cognitive symptoms of Alzheimer disease (AD) (Kalauni et al. 2002, Atta-ur-Rahman et al. 2004) and other possible therapeutic applications in the treatment of Parkinson's disease, senile dementia, and ataxia, among others (Ahmad et al. 2003a). AChE inhibitors as eserine, tacrine, donepezil, rivastigmine, and galanthamine are the only drugs currently approved for the treatment of AD; however, these drugs are known to have limitations for clinical use due to their short-half-lives and/or unfavorable side-effects (Sung et al. 2002).

Because the search of plant extracts that selectively inhibit AChE is of paramount importance to find novel and more potent AChE inhibitors, many of them have been screened and as consequence there have been found extracts and isolated pure compounds with AChE and/or butyrylcholinesterase (BChE) inhibitory properties. Among the plant families that have been examined for their AChE inhibitory activity are: Amaryllidaceae (Houghton et al. 2004, Rhee et al. 2004), Boraginaceae (Ahmad et al. 2003b), Chenopodiaceae (Ferheen et al. 2005), Lamiaceae (Ahmad et al. 2005), Liliaceae (Atta-ur-Rahman et al. 2002), and Solanaceae (Roddick 1989, Choudhary et al. 2004).

Continuing with the study of the flora from Regional Natural Park Ucumarí (RNPU, Risaralda, Colombia) as a source of new secondary metabolites with diverse grade of biological activities (Niño et al. 2003, 2006, Mosquera et al. 2004a) and the fact that there still is great interest in finding novel and better AChE inhibitors, prompted us to screen 27 crude methanol extracts belonging to the Asteraceae, Euphorbiaceae, Melastomataceae, Rubiaceae, and Solananceae families for their AChE inhibitory activities.

MATERIALS AND METHODS

In this study, the solvent hexanes, dichloromethane, and methanol (analytical-reagent grade) were purchased from Mallinckrodt (Phillipsburg, NJ, US). Silica gel plates (Silica gel 60 F_{254} 0.2 mm layer thickness) were purchased from Merck (Darmstadt, Germany). The electric eel acetylcholinesterase Sigma (St Louis, MO, US) was used. Acetylthiocholine iodide and 5,5-dithiobis (2-nitro benzoic acid) (DTNB) were purchased from Aldrich (St Louis, MO, US). A Spectronic Genesys 5 spectrophotometer Milton Roy (Rochester, NY, US) was used for all measurements of acetylcholinesterase inhibitory activity.

Plant material - Twenty seven plants from the selected families were collected at RNPU in February 2000 and October 2001, they were authenticated by Prof. FJ Roldán and are listed on the Table. Voucher specimens for each plant collected were deposited at University of Antioquia Herbarium (Medellín, Colombia).

The collected plant materials were worked out and extracted according to the procedure described by Niño et al. (2006).

In vitro acetylcholinesterase inhibition assay - In this work, 27 crude methanol plant extracts were examined for their AChE inhibitory activities at concentrations of 1000, 100, and 10 mg/l and were dissolved in a base-tris (0.05 M) buffer, following the spectrophotometric method developed by Ellman et al. (1961) as described by Salles et al. (2003). In this method, to a 1 cm path length glass cell, were added in order, 200 µl of acetylthiocholine iodide (15 mM), 1000 µl of DTNB (3 mM), and 200 µl of each test extract solution at the different concentrations evaluated, which were mixed and incubated for 15 min at 30ºC. Then, the mixture was monitored spectrophotometrically at 412 nm 10 times, each 13 s. After that, 200 µl of AChE (0.3 U/ml) solution were added to the initial mixture, to start the reaction and then the absorbance was determined.

Control contained all components except the tested

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extract. As positive control eserine (2.75 mg/l) was used. The percentage of AChE inhibitory activity (% IA) was calculated by using the following equation:

$$\% IA = [(Cc - Ce)/Cc] \times 100$$

where: Cc is the control kinetic (containing all reactants, except the AChE enzyme) and Ce is the experimental kinetic for each sample concentration. All treatments were performed in triplicate with two replicates.

Estimation of IC_{50} values - The concentrations of the tested extracts that inhibited the hydrolysis of substrate (acetylthiocholine) by 50% (IC_{50}) were determined by a linear regression analysis between the inhibition percentages against the extract concentrations by using the Excel program.

Phytochemical screening - For each plant extract a phytochemical screening was performed testing the presence of secondary metabolites by using TLC analyses. The solvent system was chloroform:ethyl acetate: methanol (2:2:1), development was performed on aluminium plates coated with silica gel 60 F_{254}. The following spray reagents were used in order to develop the spots: anisaldehyde-sulphuric acid (sterols), 1% ferric chloride (tannins), 2% aluminium chloride in ethanol (flavonoids), 1% vainillin in sulphuric acid-ethanol (saponins), and Dragendorff 's reagent (alkaloids) (Harborne 1980).

RESULTS AND DISCUSSION

The concentrations of the crude plant extracts that inhibited the enzyme activity by 50% (IC_{50}) are presented in the Table. All results exhibited a correlation coefficient of $r^2 > 0.9$. From the 27 methanol extracts evaluated only 14.81% showed strong to moderate AChE inhibitory activities.

The strongest AChE inhibitory activities were exhibited by the methanol extracts of *Schistocarpha sinforosi* Cuatrec. (IC_{50} = 145.31 mg/l) (Asteraceae), *Solanum leucocarpum* Dunal (IC_{50} = 204.59 mg/l), and *Witheringia coccoloboides* (Damm.) Hunz (IC_{50} = 220.68 mg/l), both last species belonging to Solanaceae family and *Chromolaena tequendamensis* (Hieron) R.M. King & H. Rda. (IC_{50} = 359.36 mg/l) (Asteraceae). However, the phytochemical analysis on the methanol extracts in both Asteraceae species showed the presence of polyphenols, which can generate false-positives effects (Salles et al. 2003). In addition, the evaluation of all species selected for this study by using the AChE inhibition in the TLC assay based on Ellman's method, showed that *S. sinforosi* and *C. tequendamensis* exhibited activities of false-posi-

TABLE

In vitro inhibition of acetylcholinesterase and phytochemical screening of 27 crude methanolic plant extracts from Colombian flora

Family	Species (Voucher no.)	IC_{50} (mg/l)	Alkaloids	Sterols	Saponins	Polyphenols
Asteraceae	*Chromolaena tequendamensis* (Hieron) R.M. King y H. Rda. (FJR 3730)	359.36	-	-	-	++
Asteraceae	*Liabum asclepiadeum* Sch. Bip. (FJR 3720)	> 1000	-	+	+	-
Asteraceae	*Montanoa* sp. (FJR 3749)	> 1000	-	+	-	+
Asteraceae	*Munnozia senecionidis* Benth. (FJR 3721)	> 1000	-	+	+	-
Asteraceae	*Schistocarpha sinforosi* Cuatrec (FJR 3725)	145.31	-	-	-	+
Asteraceae	*Verbesina nudipes* Blake (FJR 3746)	> 1000	-	+	+	-
Euphorbiaceae	*Acalypha diversifolia* Jacq (FJR 3726)	> 1000	+	++	++	+++
Euphorbiaceae	*Acalypha macrostachya* Jacq (FJR 3738)	> 1000	+	+	++	-
Euphorbiaceae	*Alchornea grandiflora* Mûll. Arg (FJR 3727)	> 1000	-	++	+++	++
Melastomataceae	*Miconia* sp. (FJR 3739)	> 1000	-	++	-	++
Rubiaceae	*Cinchona pubescens* Vahl (FJR 3161)	> 1000	+	-	-	-
Rubiaceae	*Diocidendron dioicum* Steyem (FJR 3748)	> 1000	-	-	-	+
Rubiaceae	*Gonzalagunia rosea* Standl (FJR 3731)	> 1000	-	-	++	++
Rubiaceae	*Hoffmannia asperula* Standl (FJR 3169)	> 1000	+	+	+	+
Rubiaceae	*Palicourea andaluciana* Standl (FJR 3183)	> 1000	+	+	+	+
Rubiaceae	*Palicourea petiolaris* Wemh (FJR 3182)	> 1000	+	+	+	+
Solanaceae	*Cestrum olivaceum* (FJR 3159)	> 1000	-	+	-	+
Solanaceae	*Deprea glabra* (Standl.) A.T. Hunz (FJR 3722)	> 1000	-	-	-	-
Solanaceae	*Lycianthes* sp. (FJR 3735)	> 1000	+	-	-	+
Solanaceae	*Lycianthes acutifolia* (R y P) (FJR 3156)	> 1000	++	+	+	-
Solanaceae	*Lycianthes radiata* (Sendt) (FJR 3154)	> 1000	++	-	-	+
Solanaceae	*Solanum aphyodendron* S. Knapp. (FJR 3729)	> 1000	+	-	-	+
Solanaceae	*Solanum lepidotum* Dunal (FJR 3728)	> 1000	+	-	-	+
Solanaceae	*Solanum leucocarpum* Dunal (FJR 3717)	204.59	+++	-	-	+
Solanaceae	*Solanum ovalifoliun* Dunal (FJR 3714)	> 1000	+	-	-	+
Solanaceae	*Solanum sycophanta* Dunal (FJR 3737)	> 1000	+	-	-	+
Solanaceae	*Witheringia coccoloboides* (Damm.) Hunz (FJR 3155)	220.68	+++	++	++	-
Eserine (2.75 mg/l)		100% inhibition				

-: absent; +: weak content; ++: moderate content; +++: strong content.

tives (data not shown) which can be attributed to the presence of aldehydes on the methanol extracts of these species (Rhee et al. 2003, Mosquera et al. 2004b). Therefore, the true AChE inhibitory activity in this work was displayed mainly by both species belonging to the Solanaceae family.

In general, in the Solanaceae family there has been reported many species with strong AChE inhibitory properties, such is the case of *Withania somnifera* where three withanolides showed AChE activity (Choudhary et al. 2004). In addition, according to Roddick (1989), the inhibitory effects of the steroidal glycoalkaloids α-solanine and α-chaconine on human AChE, showed that both alkaloids were equally active and no synergism occurred between the two compounds. On the other hand, α-solanine and α-chaconine, displayed stronger AChE inhibitory activity than the glycoalkaloids solasonine and solamargine, although they share the same trioside carbohydrate moieties. This confirms the importance of the aglycone moiety structure and evidence the fact that heterocyclic nitrogen of steroidal alkaloids plays an important feature on AChE inhibition.

The AChE inhibitory activities of *S. leucocarpum* Dunal and *W. coccoloboides* Damm. have never been reported before and their inhibitory AChE activities could be attributed to their high alkaloidal contents (see Table); this correlates with the statement established by Roddick (1989), since their AChE inhibitory activity, could be due to the presence of steroidal glycoalkaloids, which are characteristic to the Solanaceae family.

The Solanaceae, *S. leucocarpum* and *W. coccoloboide*s showed the highest AChE inhibitory activity in this study, making these two species an important target for the isolation and characterization of the phytocompounds responsible for this biological activity. This fact, confirms that the flora from RNPU has a high potential for the discovery of new and valuable compounds with diverse grade of pharmaceutical applications.

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