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Research Article

Acetylcholinesterase Inhibitory Activity of Extracts from Angolan Medicinal Plants

Fernandes, N A F[1], Canelo, L I N[2], Mendonça, D I M D de[2], Mendonça, A J G de[1*]

[1]*CICS-UBI – Health Sciences Research Centre, University of Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.*

[2]*Materiais Fibrosos e Tecnologias Ambientais – FibEnTech, University of Beira Interior, Rua Marquês d'Ávila e Bolama, 6200-001 Covilhã, Portugal.*

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ABSTRACT

Plants are considered important sources of new chemical entities that can be used in the development of novel therapeutic drugs for the symptomatic treatment of Alzheimer's disease.

The inhibition of acetylcholinesterase by 38 extracts from 13 medicinal plants used in Angola folk medicine are presented: *Adenodolichos huillensis* (roots and leaves); *Boscia microphylla* (leaves); *Croton gratissimus* (aerial part); *Gymnosporia senegalensis* (branches); *Hymenodictyon floribundum* (barks); *Parinari capensis* (leaves); *Peucedano angolense* (aerial part); *Phragmanthera glaucocarpa* (roots); *Rhus kirkii* (leaves); *Solanecio mannii* (branches); *Solanum incanum* (fruit); *Tinnea antiscorbutica* (aerial part) and *Xylopia odoratissima* (leaves). TLC bioautographic assay and Ellman's method were used. The best results for IC_{50} were obtained with the toluene extract of *B. microphylla* leaves (0.55 ± 0.01 mg/mL) and the methanol extract of *G. senegalensis* branches (0.30 ± 0.00 mg/mL). The aqueous extract of the leaves of *P. capensis* also demonstrated acetylcholinesterase inhibitory activity in the two methods used. In conclusion, the medicinal plants *P. capensis*, *B. microphylla* and *G. senegalensis* represent promising sources of natural compounds with acetylcholinesterase inhibitory properties.

Keywords - Alzheimer's disease, Acetylcholinesterase inhibitors, Ellman's method, TLC bioautographic assay, Angola folk medicine, Medicinal plants.

INTRODUCTION

The natural products obtained from plants can be used in the treatment of neurodegenerative diseases, as is the case of huperzine A obtained from *Huperzia serrata* and galanthamine obtained from *Galanthus caucasicus*, which are both used in the symptomatic treatment of Alzheimer's disease (AD)[1]. AD is a neurodegenerative disorder of the central nervous system that is characterized by cholinergic dysfunction, beta-amyloid (Aβ) plaques and neurofibrillary tangles[2].

The current treatments for AD are not capable of stopping the progression of the disease; however, patients obtain temporary relief of the symptoms by using inhibitors of the enzyme acetylcholinesterase (AChEIs)[3] and N-methyl-d-aspartate (NMDA) receptor antagonists[4]. Acetylcholinesterase (AChE, E.C. 3.1.1.7) is a serine-protease that hydrolyses the carboxylic ester of the neurotransmitter acetylcholine (ACh), which is present in neuromuscular and neuronal junctions, resulting in choline and acetic acid[5]. The structure of human acetylcholinesterase (hAChE) is identical to that of *Torpedo californica* (TcAChE)[6]. The active site of AChE is a deep and narrow gorge approximately 20 Å long that penetrates more than half-way into the enzyme and contains the "esteratic" site where the hydrolysis reaction takes place, the "anionic" site that is responsible for the binding of charged quaternary group of the choline moiety of ACh, the oxyanion hole and the acyl pocket sites that confer substrate selectivity[7]. In addition to the catalytic centre, AChE also has an additional binding site called the peripheral anionic site that is located at the narrow entrance and binds the substrate transiently as the first step in ACh hydrolysis[8]. The approved treatments for the symptomatic treatment of AD cause unwanted side effects, and their effectiveness diminishes after prolonged treatment[9]. Thus, the search for new substances for the treatment of Alzheimer's disease is warranted.

The aim of the present study was to determine the acetylcholinesterase inhibition capacity of 38 extracts from 13 plants (Table 1) used in Angolans folk medicine.

MATERIALS AND METHODS

Reagents, chemicals and equipment

Acetylcholinesterase (AChE) type V-S from the electric eel *Electrophorus electricus*, 1-naphtyl acetate, 1-naphthol, acetylthiocholine iodide (ATCI), Ellman's reagent (DTNB), fast blue B salt, tacrine, eserine, bovine serum albumin (BSA) and dimethyl sulfoxide (DMSO) were

*Author for Correspondence

Table 1: Some traditional applications and chemical constituents of selected medicinal plants from Angolans folk medicine.

Plant name (family)	*Traditional applications*	*Chemicals constituents identified*
Adenodolichos huillensis (Fabaceae)	—[a]	—[a]
Boscia microphylla (Capparaceae)	Whole plant used in the treatment of malaria[10].	—[a]
Croton gratissimus (Euphorbiaceae)	Leaves used to treat cough, chest pain and syphilis. Barks used to treat bleeding gums[11].	Cembranolides[11], lupeol and α-glutinol[12].
Gymnosporia senegalensis (Celastraceae)	Roots, leaves and barks used to treat malaria[13], tumors and snake bites[14].	Lupenone, β-sitosterol[13] and coumarins[15].
Hymenodictyon floribundum (Rubiaceae)	Barks used to treat febrile conditions[16].	Iridoids, lupenone and scopoletin[16].
Parinari capensis (Chrysobalanaceae)	Whole plant used to treat skin diseases[17] and malaria[18].	Diterpenic lactones and phytol[19].
Peucedano angolense (Apiaceae)	Whole plant used to treat chest pain[10].	—[a]
Phragmanthera glaucocarpa (Loranthaceae)	—[a]	—[a]
Rhus kirkii (Anacardiaceae)	—[a]	—[a]
Solanecio mannii (Asteraceae)	Leaves used to treat epilepsy; roots used to treat cancer, epilepsy and typhoid fever[20].	—[a]
Solanum incanum (Solanaceae)	The whole plant used to treat liver diseases[21].	Solamargine and solasonine[21].
Tinnea antiscorbutica (Labiatea)	Leaves used to prevent and treat scurvy[10].	Glutinol, friedelin, artemetin and penduletin[22].
Xylopia odoratissima (Annonaceae)	Whole plant used to treat angina[10].	—[a]

—[a] Information not found at October 2014 at ISI web of knowledge

purchased from Sigma-Aldrich (Portugal). Methanol, chloroform, ethyl acetate and toluene were purchased from Carlo Erba reagents group (France). Ethanol was purchased from Manuel Vieira & C[a] (Irmão) Lda (Portugal) and n-hexane was purchased from Valente e Ribeira Lda (Portugal). The following buffers were used: 0.1 M phosphate buffer pH 7.4 and 0.05 M Tris-HCl buffer pH 7.8. All reagents and chemicals were used without further purification, with the exception of n-hexane which was purified by distillation through a solvent distiller apparatus from Rotoquimica (Portugal). The retention factor values (Rfs) were determined using a TLC silica gel plate $60F_{254}$ from Merck (Germany). UV-Vis measurements were performed using a Pharmaspec 1700 spectrophotometer from Shimadzu (Japan) and a thermostatic bath from Thermo Scientific Haake (USA) was used to control the temperature of the cell compartment in the spectrophotometer. A rotary evaporator VV 2000 from Heidolph (Germany) was used for solvent evaporation and concentration of all plant extracts. Double deionised and filtered water (Millipore Q Advantage A10 ultra-pure water purification system; resistivity = 18.2 MΩ/cm at 25 ºC) was used for all aqueous solutions.

Plant selection and extract preparation

The medicinal plants presented in table 1 *Hymenodictyon floribundum* (barks); *Parinari capensis* (leaves); *Tinnea antiscorbutica* (aerial parts); *Xylopia odoratissima* (leaves); *Peucedano angolense* (aerial part); *Boscia microphylla* (leaves); *Adenodolichos huillensis* (roots and leaves); *Rhus kirkii* (leaves); *Croton gratissimus* (aerial part); *Gymnosporia senegalensis* (branches); *Solanecio mannii* (branches) and *Solanum incanum* (fruit) were collected in Angola in the province of Huíla and voucher specimens were deposited and identified by the staff of Lubango herbarium. The medicinal plant *Phragmanthera glaucocarpa* (roots) was collected in Angola in the province of Uíge, and a voucher specimen was deposited and identified by the staff of Luanda herbarium. After collection, the plants were dried in darkness at room temperature, except the fruit *Solanum incanum,* which was stored in methanol immediately following harvest.

Each medicinal plant sample was powdered and extracted with methanol for a week at room temperature, yielding the methanol extracts. The methanol extracts were then fractioned in chloroform, n-hexane, toluene, ethyl acetate and aqueous fractions, resulting in 38 different extracts (table 2). All plant extracts were concentrated using a rotary evaporator at 40 °C.

Acetylcholinesterase (AChE) inhibition

AChE inhibition by Ellman's colorimetric method

AChE inhibitory activity was measured using Ellman's method[23] and was performed according to Giovanni and

Table 2: Reference number of the different extracts used in AChE inhibitory activity.

Plant name	Extraction solvent (part of plant used)	Number
Adenodolichos huillensis	H_2O (leaves)	1
	$CHCl_3$ (leaves)	2
	$CHCl_3$ (roots)	3
	MeOH (leaves)	4
	Hex (leaves)	5
Boscia microphylla	PhMe (leaves)	6
	H_2O (leaves)	7
	AcOEt (leaves)	8
Croton gratissimus	Hex (aerial part)	9
	MeOH (aerial part)	10
	AcOEt (aerial part)	11
	$CHCl_3$ (aerial part)	12
	H_2O (aerial part)	13
Gymnosporia senegalensis	MeOH (branches)	14
Hymenodictyon floribundum	MeOH (barks)	15
	H_2O (barks)	16
	$CHCl_3$ (barks)	17
	Hex (barks)	18
Parinari capensis	Hex (leaves)	19
	MeOH (leaves)	20
	H2O (leaves)	21
	$CHCl_3$ (leaves)	22
Peucedano angolense	MeOH (aerial part)	23
	H_2O (aerial part)	24
	$CHCl_3$ (aerial part)	25
Phragmanthera glaucocarpa	Hex (roots)	26
	MeOH (roots)	27
	AcOEt (roots)	28
	$CHCl_3$ (roots)	29
	H_2O (roots)	30
Rhus kirkii	MeOH (leaves)	31
Solanecio mannii	MeOH (branches)	32
Solanum incanum	MeOH (fruit)	33
	AcOEt (fruit)	34
	H_2O (fruit)	35
Tinnea antiscorbutica	MeOH (aerial part)	36
	H_2O (aerial part)	37
Xylopia odoratissima	PhMe (leaves)	38

MeOH- methanol; Hex- hexane; CHCl3- chloroform; AcOEt-ethyl acetate; H2O- water; PhMe- toluene.

collaborators[24], with minor modifications. Briefly, quartz cuvettes were filled with 421 µl of 0.15 mM Ellman's reagent (in 0.1 M phosphate buffer pH 7.4), 37 µL of a 0.037 U/mL solution of AChE (in 0.1 M phosphate buffer pH 7.4) and 5 µL of plant extract solutions prepared in dimethyl sulfoxide (DMSO) or methanol at concentrations between 0.01 mg/mL and 50 mg/mL. Cuvettes were allowed to incubate for 5 min at 37 °C. The enzymatic reaction was started by the addition of 37 µL of acetylthiocholine iodide (ATCI) prepared in type I water to a final concentration equal to the K_m value of AChE, which was previously determined according to Giovanni and collaborators[24]. The K_m values obtained were 0.065 mM and 0.191 mM using 5 µL of DMSO or methanol, respectively. The final assay volume was 500 µL and the increase in absorbance at 412 nm was monitored at 37 °C for 3 min using a UV-Vis spectrophotometer with a thermostated cell compartment. The blank consists of 5 µL of DMSO or methanol in the cuvette instead of the extract solution. A solution of tacrine prepared in DMSO or methanol at concentrations between 0.01 mg/mL and 50 mg/mL was used as a reference. The AChE inhibition activity (%) was calculated by equation (1):

$$\text{AChE Inhibition (\%)} = [(A_B - A_S) / A_B] \times 100 \quad (1)$$

where A_B is the variation in the absorption of the blank sample in the time 0 s – 180 s, and A_S is the variation in the absorption of the test sample extract in the same time. The test sample concentration that caused 50 % inhibition of AChE activity (IC_{50}) was calculated from the curve in which AChE inhibition (%) was plotted *vs* sample concentration (mg/mL). The extract samples were dissolved in methanol or DMSO according to their solubility in the solvent and reaction medium.

AChE inhibition by thin layer chromatography (TLC) bioautography assay

The thin layer chromatography (TLC) bioautographic

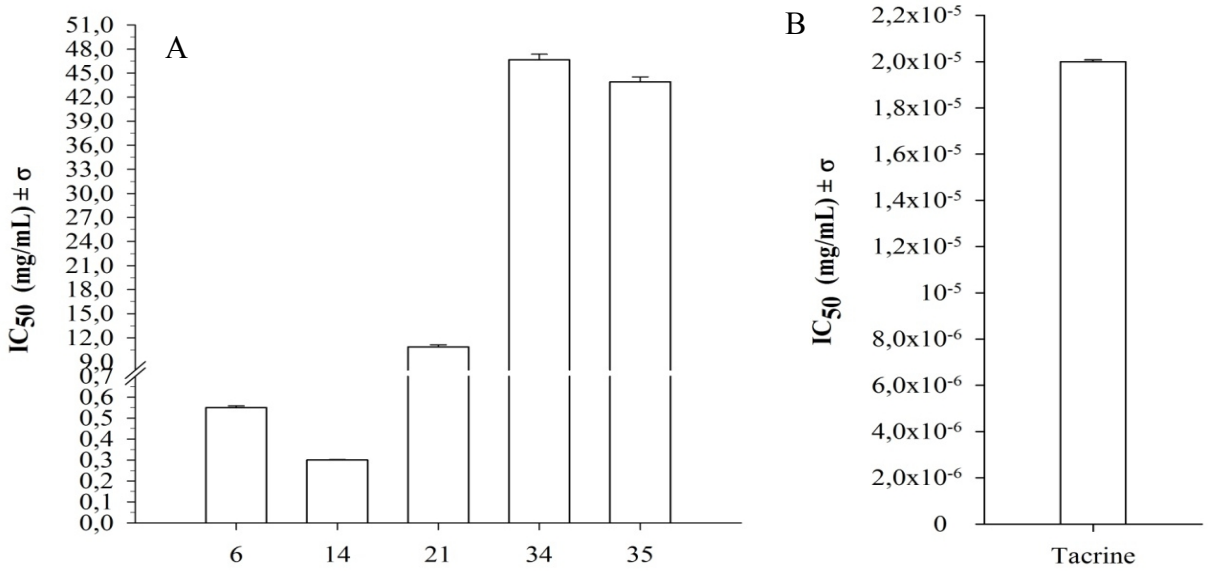

Figure 1: AChE inhibition represented by the IC_{50} values determined through Ellman's method for samples dissolved in dimethyl sulfoxide (A) and for tacrine dissolved in dimethyl sulfoxide (B). The numbers on the abscissa correspond to samples identified in table 2. (n = 3; mean ± σ).

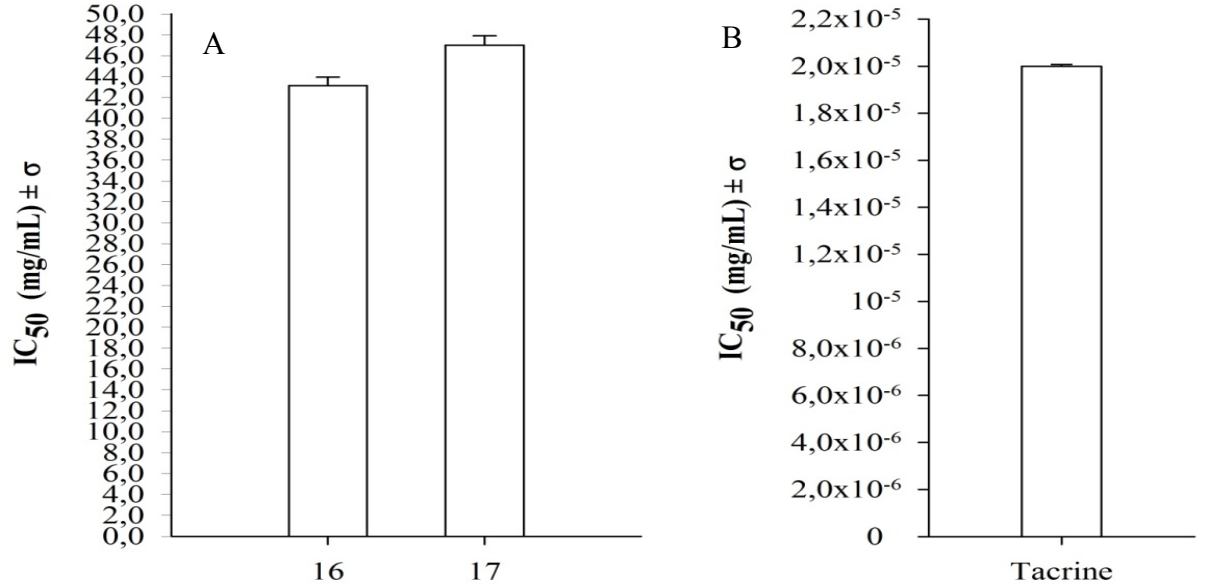

Figure 2: AChE inhibition represented by the IC_{50} values determined through Ellman's method for samples dissolved in methanol (A) and for tacrine dissolved in methanol (B). The numbers on the abscissa correspond to samples identified in table 2. (n = 3; mean ± σ).

assay was performed according to Marston and collaborators[25], with minor modifications. A solution of AChE 30 U/mL was prepared in 0.05 M Tris-HCl buffer pH 7.8 with 1 mg/mL bovine serum albumin (BSA). A working solution of AChE 6.7 U/mL was prepared by dilution with the Tris-HCl buffer and the solutions of 1-naphthyl acetate (2.5 mg/mL) and fast blue B salt (2.5 mg/mL) were prepared in ethanol and type I water, respectively. Eserine was used as standard compound, and to set the minimum concentration of eserine that produced a visible spot on the chromatographic plate, concentrations of eserine between 0.001 mg/mL and 0.100 mg/mL were used. Fifteen microliters of extract test samples (5 mg/mL) and eserine (0.100 mg/mL) were applied to a silica gel TLC plate and eluted with the appropriate mixture of solvents. Previous elution tests of the samples on the TLC plate were carried out to determine the solvents and respective proportions to obtain maximum separation of the components of each sample extract. After elution of the samples the plate was completely dried and sprayed with AChE working solution. The TLC plate was then maintained in a sealed plastic box containing type I water for 20 min at 37 °C, without direct contact with the water, after which the plate was partially dried. Next, the TLC plate was sprayed with 1-naphthyl acetate (2.5 mg/mL) and after incomplete drying of the plate, fast blue B salt (2.5 mg/mL) was sprayed onto the TLC plate. White spots appear due to the inhibited acetylcholinesterase, standing out from the purple background. To ensure that the white spots were not a false positive, a test for the detection of

Table 3: Percentage of inhibition of AChE determined by Ellman's method for the different extracts dissolved in methanol or DMSO, for a maximum sample concentration. (n= 3; mean ± σ).

Samples	Concentration (mg/mL)	DMSO	MeOH
		AChE Inhibition (% ± σ)	AChE Inhibition (% ± σ)
1	0.5	15.29 ± 0.52	6.29 ± 0.13
2	0.01	ND	ND
3	0.5	10.82 ± 0.65	ND
4	0.01	12.57 ± 0.68	0.37 ± 0.02
5	0.1	28.24 ± 0.95	ND
6	0.1	69.74 ± 1.54	ND
7	0.5	27.10 ± 1.39	ND
8	0.5	43.63 ± 1.27	ND
9	0.5	40.92 ± 1.89	48.29 ± 0.91
10	0.5	43.74 ± 1.15	25.33 ± 1.50
11	0.5	16.58 ± 1.14	17.40 ± 0.28
12	0.5	30.43 ± 1.10	10.46 ± 0.27
13	0.1	1.83 ± 0.07	ND
14	0.5	76.14 ± 1.70	44.84 ± 1.23
15	0.01	ND	34.61 ± 0.10
16	0.5	43.82 ± 1.91	47.94 ± 1.75
17	0.5	ND	39.40 ± 1.21
18	0.5	16.30 ± 0.90	18.17 ± 0.92
19	0.01	ND	ND
20	0.01	32.91 ± 1.29	ND
21	0.1	49.37 ± 1.15	33.97 ± 1.51
22	0.01	ND	9.31 ± 0.43
23	0.5	36.65 ± 1.29	19.88 ± 0.27
24	0.01	15.69 ± 1.05	13.20 ± 1.29
25	0.01	6.76 ± 0.37	23.39 ± 1.15
26	0.5	ND	3.64 ± 0.12
27	0.5	ND	38.81 ± 1.01
28	0.5	ND	42.92 ± 1.07
29	0.5	ND	48.07 ± 1.26
30	0.5	ND	46.08 ± 1.48
31	0.01	ND	8.85 ± 0.94
32	0.01	ND	5.83 ± 0.11
33	0.5	27.56 ± 1.90	7.38 ± 0.54
34	0.5	48.29 ± 1.14	10.32 ± 0.38
35	0.5	43.39 ± 1.61	15.78 ± 0.40
36	0.5	ND	47.15 ± 1.56
37	0.5	ND	17.67 ± 0.70
38	0.1	ND	1.33 ± 0.02
Tacrine	0.5	96.45 ± 1.87	96.54 ± 0.81

ND - Not determined, due to the extract precipitation at the solvent or reaction medium used.

false positives was carried out according to Yang and collaborators[26]. Thus, a solution of 1-naphthol 1.5 mg/mL was prepared using a mixture of ethanol (20 mL) and type I water (30 mL). Aliquots (15 µL) of the extracts (5 mg/mL) were applied to the TLC plate and eluted with the appropriate mixture of solvents. After the elution, the plate was completely dried and the solution of 1-naphthol was sprayed. The plate was dried once again and fast blue B salt (2.5 mg/mL) was sprayed to TLC plate. The appearance of white spots indicated a false positive.

Statistical analysis

All determinations were conducted in triplicate for each test sample, and the results presented throughout the text and tables represent the mean ± standard deviation (σ). The IC_{50} values were calculated by linear regression analysis using Microsoft Excel 2013, and figures were obtained using the Sigmaplot program release 11.0 (Systat Software Inc., USA).

RESULTS AND DISCUSSION

Medicinal plant extracts

The 38 different extracts obtained from the 13 plants and used to evaluate the AChE inhibition are listed in table 2. For each extract sample, a numerical code was assigned (see table 2).

AChE inhibition by Ellman's method and TLC bioautography assay

The percentage of inhibition of acetylcholinesterase is given by equation (1). The IC_{50} values from the different samples dissolved in DMSO and methanol were then

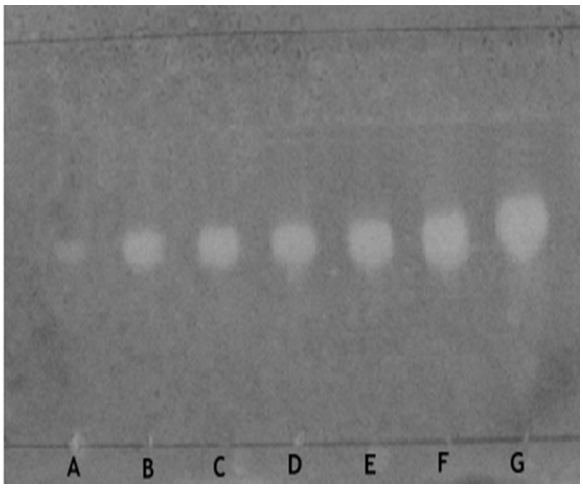

Figure 3: Chromatographic plate obtained through the TLC bioautographic method for different concentrations of eserine. A - 0.001 mg/mL, B - 0.010 mg/mL, C - 0.015 mg/mL, D - 0.020 mg/mL, E - 0.025 mg/mL, F - 0.050 mg/mL and G - 0.100 mg/mL (80:20 $CHCl_3$ - MeOH). For all the spots the retention factor obtained was Rf = 0.55.

determined by interpolation on the curves of AchE. The determination of the AChE kinetic parameters (K_m and V_{max}) at 37 ºC, in the presence of 1 % methanol or DMSO, was performed through the linearization of Lineweaver-Burk. For 1 % methanol, the K_m obtained was 0.19 ± 0.01 mM and the V_{max} was $8.08 \times 10^{-3} \pm 2.00 \times 10^{-4}$ mM^{-1}.s. For 1 % DMSO the K_m was 0.07 ± 0.01 mM and the V_{max} was $1.49 \times 10^{-3} \pm 1.75 \times 10^{-4}$ mM^{-1}.s. K_m and V_{max} values obtained with DMSO were lower than the values obtained with methanol, which confirms that DMSO affects the activity of the enzyme acetylcholinesterase[24]. The AChE inhibitor tacrine dissolved in methanol or DMSO, as appropriate, was used as a reference compound in Ellman's method. The IC_{50} for tacrine dissolved in DMSO and methanol was 0.02 ± 0.00 µg/mL in both solvents. From the 38 extracts it was only possible to determine the IC_{50} for samples number 6 (toluene extract of the leaves of *B. microphylla*), 14 (methanol extract of the branches of *G. senegalensis*), 16 (aqueous extract of the bark of *H. floribundum*), 17 (chloroform extract of the bark of *H. floribundum*), 21 (aqueous extract of the leaves of *P. capensis*), 34 (ethyl acetate extract of the fruit of *S. incanum*) and 35 (aqueous extract of the fruit of *S. incanum*). For all other extract samples it was not possible to calculate the IC_{50} due to the lack of solubility in the reaction medium or in the solvent when used for higher concentrations. In Ellman's method, extract precipitation at the higher concentrations was the main reason for the limited number of samples in the IC_{50} determination. From the seven samples, only five presented solubility in DMSO and reaction medium in the cuvette (figure 1) and two were only soluble in MeOH and the reaction medium (figure 2). A low IC_{50} value is indicative of good inhibition of acetylcholinesterase. The IC_{50} values obtained for the seven samples are higher than the IC_{50} value for tacrine (figures 1 and 2). However, from the seven samples, it was observed that the best results were obtained from two extract samples dissolved in DMSO, the toluene extract of

Table 4: Results of AChE inhibition by TLC bioautographic method

Samples	Rfs	Solvents proportions (% v/v)
2	0.46	80:20 ($CHCl_3$ - MeOH)
5	0.26	80:20 (Hex - AcOEt)
6	0.26	
9	0.26	
	0.44	
	0.72	
10	0.25	
	0.45	
11	0.59	95:5 (AcOEt-MeOH)
12	0.49	80:20 ($CHCl_3$-MeOH)
	0.61	
	0.75	
18	0.26	80:20 (Hex-AcOEt)
	0.67	
19	0.26	
21	0.78	70:30 ($CHCl_3$-MeOH)
23	0.25	80:20 (Hex-AcOEt)
29	0.54	80:20 ($CHCl_3$-MeOH)
	0.61	
32	0.25	80:20 (Hex-AcOEt)
38	0.26	
	0.44	

MeOH- methanol; Hex- hexane; $CHCl_3$- chloroform; AcOEt-ethyl acetate.

the leaves of *B. microphylla* (sample 6) with an IC_{50} of 0.55 ± 0.01 mg/mL and the methanol extract of the *G. Senegalensis* branches (sample 14) with an IC_{50} of 0.30 ± 0.00 mg/mL. The IC_{50} value of the methanol extract of the branches of *G. senegalensis* was the lowest, indicating that the branches of this plant contain compounds that are highly capable of inhibiting AChE activity, beyond the effects of DMSO in the enzyme acetylcholinesterase. Because it was not possible to determine the IC_{50} for a large number of samples due to the lack of solubility, the values of AChE inhibition (%) (table 3) were determined for a 0.5 mg/mL extract concentration, with the exception of the cases in which the extracts were not soluble. In those cases, the extract concentration presented was at the maximum value to which solubility was verified. In table 3, for extracts dissolved in DMSO, the best results of AChE inhibition were obtained from samples number 14 (methanol extract of the branches of *G. senegalensis*) with 76.14 ± 1.70 %, 6 (toluene extract of the leaves of *B. microphylla*) with 69.74 ± 1.54 %, 21 (aqueous extract of the leaves of *P. capensis*) with 49.37 ± 1.50 % and 34 (ethyl acetate extract of the fruit of *S. incanum*) with 48.29 ± 1.14 %. For extracts dissolved in methanol, the best inhibition (%) versus sample concentration (mg/mL) (AChE inhibition by Ellman's colorimetric method) results of AChE inhibition were obtained from samples number 9 (hexane extract of the aerial part of *C. gratissimus*) with 48.29 ± 0.91 %, 29 (chloroform extract of the roots of *P. glaucocarpa*) with 48.07 ± 1.26 %, 16 (aqueous extract of the barks of *H. floribundum*) with 47.94 ± 1.75 % and 36 (methanol extract of the aerial part of *T. antiscorbutica*)

with 47.15 ± 1.56 %. The differences in the results presented in the table 3 can be explained by the influence of dimethyl sulfoxide and methanol, respectively, on the acetylcholinesterase activity[24]. TLC bioautographic assay was used to evaluate the ability of the different extract samples to inhibit acetylcholinesterase, thus eliminating the interference of solvents in the inhibition results of the different extracts. In the samples in which inhibition was observed on the TLC plate, the retention factor (Rf) of the respective spot was calculated (table 4). The AChE inhibition spots appeared as white spots on a purple background (figure 3, shown in greyscale). To establish detection limits for the bioautographic assay, the acetylcholinesterase inhibitor eserine was applied at various concentrations onto the TLC plate (figure 3), and the lowest concentration with an observable white spot was determined. The minimum concentration of eserine at which it was possible to detect a white spot of inhibition was 0.001 mg/mL (figure 3, shown in greyscale). Among the 38 extracts tested, only 14 extracts showed inhibition of acetylcholinesterase activity. For some extracts, the inhibition of AChE is due to more than one compound present in the extract (table 4). The samples with two retention factors (spots for AChE inhibition) were the hexane extract of the bark of *H. floribundum* (sample 18), the toluene extract of the leaves of *X. odoratissima* (sample 38), the methanol extract of the aerial parts of *C. gratissimus* (sample 10) and the chloroform extract of the roots of *P. glaucocarpa* (sample 29). Samples with three spots for AChE inhibition were the hexane extract of the aerial parts of *C. gratissimus* (sample 9) and the chloroform extract of the aerial parts of *C. gratissimus* (sample 12). For samples number 2 (chloroform extract of the leaves of *A. huillensis*), 5 (hexane extract of the leaves of *A. huillensis*), 6 (toluene extract of the leaves of *B. microphylla*), 11 (ethyl acetate extract of the aerial parts of *C. gratissimus*), 19 (hexane extract of the leaves of *P. capensis*), 21 (aqueous extract of the leaves of *P. capensis*), 23 (methanol extract of the aerial parts of *P. angolense*) and 32 (methanol extract of the branches of *S. mannii*), only one spot for AChE inhibition was observed. However, this does not mean that the result is due to a single compound; different compounds in the extracts with close retention factors may exist, thus causing overlapping spots. The inactivation of the compounds after interaction with the silica of the chromatographic plate and the conformational change of the enzyme after adsorption on the plate may explain why the samples with positive results in Ellman's method do not show AChE inhibition by the TLC bioautographic method[24]. Additionally, the conformational change of the enzyme may, in some cases, facilitate the interaction between the enzyme and the inhibitor or cause difficulty for this interaction, which leads to the different results between the two methods used for evaluation of AChE inhibition[24]. The extracts from the plants *A. huillensis*, *B. microphylla*, *P. angolense*, *P. glaucocarpa*, *S. mannii* and *X. odoratissima* have AChE inhibitory activities (tables 3 and 4), but their chemical composition is not yet known. Thus, the separation, purification and identification of the compounds present in the extracts of these plants are crucial to determine which compound or compounds are responsible for the inhibition of acetylcholinesterase. In contrast, the extracts from the plants *C. gratissimus*, *G. senegalensis*, *H. floribundum*, *P. capensis* and *S. incanum* have AChE inhibitory activities (tables 3 and 4), and the chemical composition of these plants is known (table 1). The plants *C. gratissimus*, *G. senegalensis*, *H. floribundum* and *S. incanum* have in their composition lupeol, coumarins, scopoletin and solamargine, respectively (table 1). These compounds exhibit AChE inhibitory activity[27-30] and the AChE inhibition by the extracts of these plants could be attributed to the presence of these and other compounds in its chemical composition. For *P. capensis*, no AChE inhibition studies have been conducted for the compounds presents in this plant.

CONCLUSIONS

The results of the present study indicate that the toluene extract of *Boscia microphylla* leaves and the methanol extract of *Gymnosporia senegalensis* branches contain chemical compounds with good AChE inhibitory activity. Thus, the toluene and methanol extracts of these two medicinal plants can be regarded as promising sources of natural compounds with cholinesterase inhibitory activity. For the other extracts with AChE inhibitory activity, it will be necessary to separate, purify and identify the pure compounds obtained, before further experiments are conducted because the isolated of pure compounds may lead to better results in regard to the inhibition of acetylcholinesterase. The aqueous extract of the leaves of *P. capensis* can also be regarded as a promising source of natural compounds with AChE inhibition because the aqueous extract of this medicinal plant presented AChE inhibitory activity in the two methods used to evaluate acetylcholinesterase inhibition.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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