ANTICHOLINESTERASE ACTIVITY OF ENDEMIC PLANT EXTRACTS FROM SOQOTRA

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Abstract:

A total of 30 chloroform and methanol extracts from the following endemic Soqotran plants *Acridocarpus socotranus Olive*, *Boswellia socotranao* Balf.fil, *Boswellia elongata* Balf. fil., *Caralluma socotrana* N. Br, *Cephalocroton socotranus* Balf.f, *Croton socotranus* Balf. fil.., *Dendrosicycos socotrana* Balf.f., *Dorstenia gigas* Schweinf. ex Balf. fil., *Eureiandra balfourii* Cogn. & Balf. fil*., Kalanchoe farinaceae Balf.f*, *Limonium sokotranum (Vierh) Radcl. Sm)*, *Oldenlandia pulvinata*, *Pulicaria diversifolia*(Balf. and *Pulicaria stephanocarpa* Balf. were screened for their acetylcholinesterase inhibitory activity by using *in vitro* Ellman method at 50 and 200 µg/ml concentrations. Chloroform extracts of *Croton socotranus, Boswellia socotrana, Dorstenia gigas,* and *Pulicaria stephanocarpa* as well as methanol extracts of *Eureiandra balfourii* exhibited inhibitory activities higher than 50 % at concentration of 200 µg. At a concentrations of 50 µg, the chloroform extract of *Croton socotranus* exhibited an inhibition of 40.6 %.

Key words: plant extracts, acetylcholinesterase inhibitors, Soqotra, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a progressive neurological disorder leading to impairment of memory and behavioral disturbances. Epidemiological data indicate that up to 4 million people are affected in the USA. AD is accompanied with cholinergic deficit in the brain, resulting in decrease in acetylcholine level (Zarotsky et al 2003). The inhibition of the acetylcholinesterase, which is responsible for the hydrolysis of acetylcholine, represents the most effective approach for finding of new anti AD compounds from natural sources. Tacrine, donepezil, rivastigmine and galanthamine with AChE inhibitory activity are used for treating mild type of AD but they possess some side effects which limits their use (Zarotsky, et al 2003, Melanie-Jayne et al 2003, Schneider, 2001).

Plants still continue to be almost the exclusive source of drugs which have been shown to contain potential new AChE inhibitors. A number of reports concerning the anticholinesterase screening of plant extracts has been published in the literature (Howes et al 2003, Melanie-Jayne et al 2003, Houghton, et al 2006) Extracts from the endemic flora of Soqotra Island, which is a home to 404 endemic plants of about 850 plants (Miller, and Morris, 2004) may represent a valuable source of new secondary metabolites with high diverse biological activities, in particular for finding new natural AChE inhibitors.

In this paper , we studied thirty extracts prepared from different parts of fourteen endemic Soqotran plants for their AChE –inhibitory activity using Ellman's colorimetric method in 96-welled microplate.

Materials and Methods

Plant materials

The plant materials were collected in March 2006 from different locations on Soqotra Island (Table 1). The plants were taxonomically identified at Soqotra Archipelago Conservation and Development Program (SCDP), Yemen. Species names are according to International Plant Name Index (IPNI) (http://www.ipni.org). Voucher specimens of the plant material were deposited at the Pharmacognosy Department, Aden University, Yemen.

Preparation of extracts

Air-dried and powdered plant materials (10 g) were extracted under shaking at room temperature successively with $CHCl_3$ (4 x 100 ml), followed by MeOH (4 x 100 ml). The obtained extracts were filtered, and evaporated to dryness *in vacuo* at 40 °C. The resulting crude extracts were stored at 4 °C.

Chemicals

Acetylthiocholine iodide (ATCI), 5,5' –dithiobis-(2-nitrobenzoic acid) (DTNB), galanthamin, bovine serum albumine (BSA) and acetylcholinesterase from horse serum (lyophilized, 500 U/vial solid, 65 U/mg) were purchased from Sigma (Germany). The following buffers were used: Buffer A: 50 mM Tris-HCl, pH 8, containing 0.1% BSA; Buffer B: 50 mM Tris-HCl, pH 8 containing 0.1 M NaCl, 0.02 M $MgCl_2 \times 6H_2O$

Microplate assay for AChE inhibitory activity

AChE inhibitory activity was detected by a microtitre plate assay based on Ellman's method (Ellman et al 1961, Rhee, et al 2001), using acetylthiocholine as a substrate. In 96-well plates, 25 µl of 15 mM ATCI, (43 mg/ 10 mL Millipore water), 125 µl of 3 mM DTNB, (11.9 mg/ 10 mL buffer B), 50 µl of buffer A and 25 µl of plant extract at concentration of 2, and 0.5 mg/ml (final concentration in the assay: 0.2, 0.05 mg /ml DMSO) were added and the absorbance was measured at 405 nm every 13 s for five times. After adding 25 µl of 0.22 U/ml enzyme, (0.34 mg AChE dissolved in 100 mL buffer A), the absorbance was read again every 13 s for five times. The absorbance was measured using a Tecan GeniosPro micro plate reader (Tecan Group Ltd , Switzerland). Percentage of inhibition was calculated by comparing the rates for the sample to the blank (DMSO), control contained all components except the tested extract, galanthamine was used as positive control. The mean of four measurements for each concentration was determined (n=4).

Results and Discussion

Fourteen endemic plant species belonging to eleven plant families were collected from Soqotra island in Yemen and a total of thirty extracts were screened for AChE inhibitory activity using Ellman's colorimetric method in 96-welled microplate, Table 1 gives the names of the plants investigated, their voucher specimen no, their families, and their traditional uses. The results obtained in two concentrations of all plant extracts are shown in Table 2.

At the concentration of 200 µg/ml, the chloroform extracts from the bark of *Croton socotranus*, resin of *Boswellia socotranus*, leaves of *Dorstenia gigas*, leaves of *Pulicaria stephanocarpa* exhibited AChE inhibitory activity with more than 50% (with percent inhibition of 79.23, 71.2, 65.1, and 61.4, respectively). The methanol extract from tuber of *Eureiandera balfouri* showed at the same concentration an AChE inhibitory activity of 58.61%. The rest of the extracts showed an AChE inhibitory activity below 50%. Galanthamine was used as standard AChE inhibitor (positive control). At the concentration of 8 µg/ml galanthamine exhibited an AChE inhibitory activity of 87.43 %. At the concentration of 200 µg/ml, which was the concentration of the test extracts in this study, galanthamine showed complete inhibition of the enzyme activity.

In this study it was found that the AChE inhibitory activity (71.2%) of resin of *B. socotrana* was more than the inhibitory activity (46.34%) of the resin from *B. elongata.*. An inhibitory activity has been reported for 11-hydroxy –beta-boswellic acid isolated from resins of *Boswellia carterii*. (Ota, and Houghton, 2005). The occurrence of boswellic acids is well documented in resins of the genus *Boswellia*, and the quantitative concentrations of the active 11-hydroxy –beta-boswellic acid in the species of the genus can be different, which might explain the observed difference in AChE inhibitory activity between *B. socotrana* and *B. elongata.*

Currently no AChE inhibitory activity has been reported from the leaves of *D. gigas.* Phytochemical investigations of *D. gigas* leaves revealed the occurrence of furanocoumarins (Franke, et al 2001), a group of compounds reported to have AChE inhibitory activity (Kang, et al 2001, Miyazawa, et al 2004). The chloroform extract from *P. stephanocarpa* leaves showed moderate AChE inhibitory activity (**61.43 %** enzyme inhibition) more than the methanolic extract. (23.21 % Enzyme inhibition). This higher AChE inhibitory activity can be explained by the higher content of essential oil with monoterpenoids, a group of compounds (e.g 1, 8-cineole, α- pinene,) reported to have AChE inhibitory activity (Houghton, et al 2006).
Croton socotranus species exhibited the highest AChE inhibitory activity in our study. According to databases, the Genus *Croton* comprises about 3100 epithets, under which only a few were investigated such as *Croton hemiargyreus* which contained alkaloids of berberine type that possessed AChE-inhibitory activity (Amaral, and Barnes, 1998). Further studies are necessary for the isolation and characterization of the active compound(s) from the active extract of C. *socotranus*.

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Table 1: list of plants tested and their traditional use

Botanical name	Collection number	Parts tested	Plant family	Traditional uses
Acridocarpus socotranus Olive	*SPM01*	L	Malpighiaceae	Headaches[2]
Boswellia socotranao Balf.fil	SPBs-03	Re	Burseraceae	nervous disorders[2]
Boswellia elongata Balf. fil.	SPe-03	Re	Burseraceae	nervous disorders[2]
Caralluma socotrana N. Br	SPAs-0.3	Ap	Asclepediaceae	dyspepsia[2]
Cephalocroton socotranus Balf.f	SPEu 05	B	Euphorbiaceae	to perfume the air[1]
Croton socotranus Balf. fil..	*SP-Eu09*	B	Euphorbiaceae	healing wounds[1]
Dendrosicycos socotrana Balf.f.	SP –C03	B,L	Cucurbitaceae	Problems of liver[1]
Dorstenia gigas Schweinf. ex Balf. fil.	.SP Mo03	L	Moraceae	Skin diseases[1]
Eureiandra balfourii Cogn. & Balf. fil.	SP –C05	T	Cucurbitaceae	unknown
Kalanchoe farinaceae *Balf.f*	*SP-Cr08*	L	Crassulaceae	ulcers[1]
Limonium sokotranum *(Vierh) Radcl.Sm)*	SP-Pl05	L	Plumbaginaceae	Antifungal[2]
Oldenlandia pulvinata	SP-Ru11	Ap	Rubiaceae	Flavoring[2]
Pulicaria diversifolia(Balf. *f*	SP-Co12	Ap	Compositae	Antispasmodic[2]
Pulicaria stephanocarpa(Balf.	SP –Co13	L	Compositae	Headache[2]

L: leaves; F: fruits; T: tubers; Ap, aerial parts Re, resins B, bark, , R, roots.
[1]Most of the ethnomedical information has been taken from (Miller and Morris 2004), and from [2]the local inhabitants

Table 2: Screening of endemic Soqotran plants for acetylcholinesterase inhibitory activity

Botanical name	Plant part tested	Extract yield[a] (%)	Extraction solvent	AChE inhibition (%)	
				0.2mg/ml	0.05mg/ml
Acridocarpus socotranus	leaves	((5.3)	$CHCl_3$	23.68	2.13
		(15.5)	MeOH	43.12	13.45
Boswellia socotranao	Resin	(51.2)	$CHCl_3$	**71.21**	22.32
		(43.21)	MeOH	35.23	0
Boswellia elongatao	Resin	(47.2)	$CHCl_3$	46.34	11.23
		(37.34)	MeOH	17.87	0
Caralluma socotrana	Aerial parts	(5.5)	$CHCl_3$	45.34	15.29
		(13.4)	MeOH	15.3	0
Cephalocroton socotranus	Bark	(6.23)	$CHCl_3$	**51.1**	14.35
		(1.03)	MeOH	36.21	9.05
Croton socotranus.	Bark	(1.81)	$CHCl_3$	**79.23**	**40.61**
		(4.61)	MeOH	13.23	0
Dendrosicycos socotrana	Bark	(1.91)	$CHCl_3$	23.35	3.21
		(5.72	MeOH	31.95	5.4
Dendrosicycos socotrana	leaves	(7.23.)	$CHCl_3$	18.35	0
		(12.12	MeOH	31.95	5.4
Dorstenia gigas	Leaves	(2.54)	$CHCl_3$	**65.12.**	18.51
		(6.35)	MeOH	26.34	7.08
Eureiandra balfourii	Tuber	(1.04)	$CHCl_3$	33.34	23.05
		(3.51)	MeOH	**58.61**	19.42
Kalanchoe farinaceae	Leaves	(5.3)	$CHCl_3$	45.21	17.02
		(12.7)	MeOH	32.23	10.56
Limonium sokotranum	Leaves	(4.61)	CHCl	43.23	15.13
		(9.51)	MeOH	29.2	12.45-
Oldenlandia pulvinata	Aerial parts	(3.91)	$CHCl_3$	45.34	16.56
		9.21	MeOH	35.67	12.12
Pulicaria diversifolia	Aerial parts	(3.61)	$CHCl_3$	41.23	9.71
		(11.31	MeOH	35.3	11.34
Pulicaria stephanocarpa	Leaves	(2.30)	$CHCl_3$	**61.43**	10.73
		(5.91)	MeOH	23.21	5.51
Galanthamine (8 µg/ml)				**87.8**	

[a]Percentage extract yield (w/w) was estimated as dry extract weight/dry starting material weight x 100

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