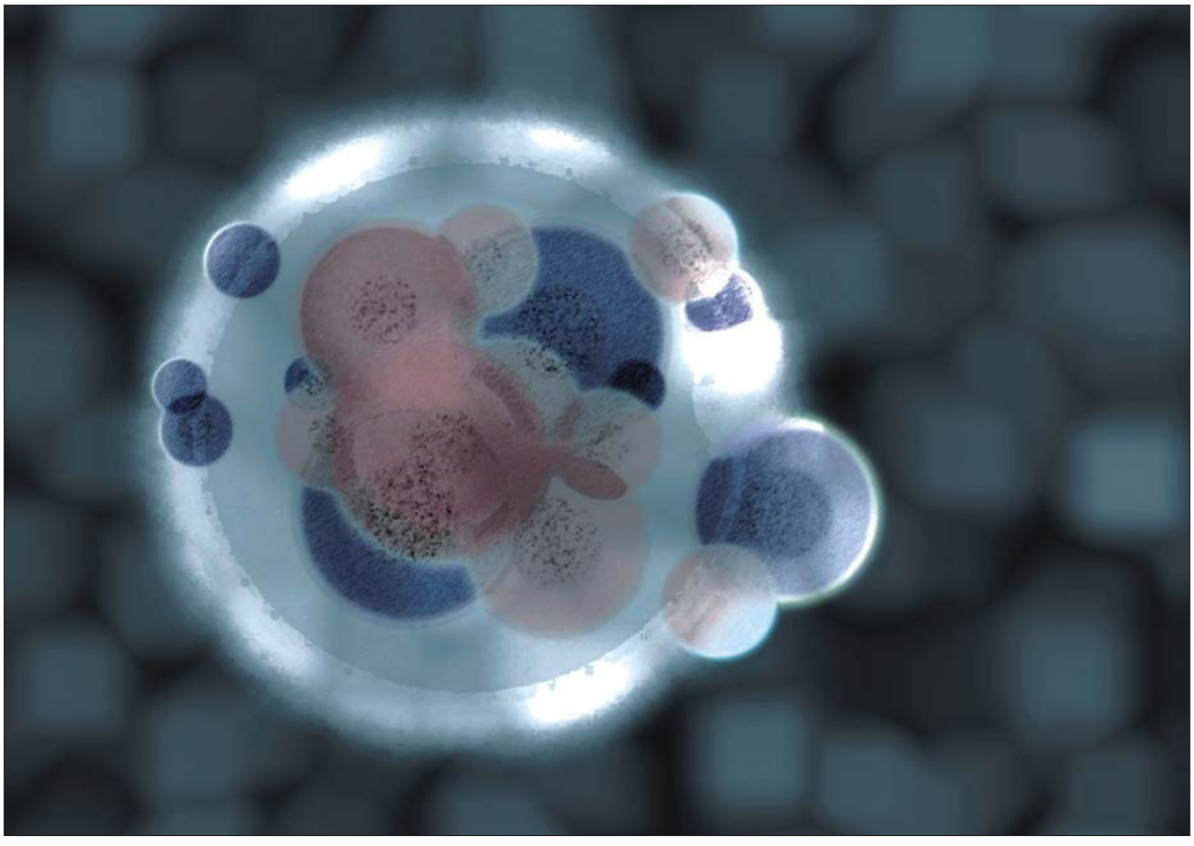

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 10 (1): 1-9, 2010
ISSN 1727-3048

Screening of Algerian Medicinal Plants for Acetylcholinesterase Inhibitory Activity

[1]H. Benamar, [1]W. Rached, [2]A. Derdour and [1]A. Marouf
[1]Laboratoire de Biochimie Végétale et des Substances Naturelles,
Département de Biologie, Faculté des Sciences
[2]Laboratoire de Synthèse Organique Appliquée, Département de Chimie,
Faculté des Sciences, Université d'Oran, BP 1524, 31000 Oran El-Menouar, Algeria

Abstract: The aim of this study was to evaluate acetylcholinesterase (AChE) inhibitory activity and phytochemical profile of some Algerian medicinal plants. The bioautography on Thin-Layer Chromatography (TLC) reveals 10 active aqueous extracts from a total of 77 extracts. Among them, aqueous extract of *Pistacia lentiscus* presents seven active spots. The Ellman's colorimetric method shows that aqueous extract of *Pistacia atlantica* and *P. lentiscus* present a strong AChE inhibition. The chloroformic fraction obtained after liquid-liquid partition of *Atriplex halimus* roots aqueous extract, presents a strong AChE inhibition among all fractions tested with IC_{50} of 9.55 μg mL^{-1}. The quantitative dosage of total phenolic compounds and total flavonoids by colorimetric method shows that *Osyris quadripartita* and *Pistacia atlantica* are the richest in total phenolic compounds (438.99 and 407.68 mg g^{-1}, respectively), a highest content on total flavonoids was detected in extract from *Rosmarinus officinalis* and *Acacia raddiana* (125.70 and 115.37 mg g^{-1}, respectively).

Key words: Acetylcholinesterase inhibition, Algerian medicinal plants, bioautography, phenolics, flavonoids

INTRODUCTION

Great level of attention has been focused on herbs and spices as sources of drugs. Algerian flora is very rich and a great number of species has been used traditionally for the treatment of several diseases without any scientific background. Indeed, many of these species are chemically unknown and may be dangerous for health. In the same time, other species not yet used by folk medicine may be potentially interesting.

Alzheimer's Disease (AD) is frequent in elderly people, as a result of malfunctioning of different biochemical pathways. According to the cholinergic hypothesis memory impairments in patients suffering from AD is a result of decreased levels of the neurotransmitter acethylcholine (ACh) in the cortex (Lahiri *et al*., 2002). The principal role of acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of ACh (Mukherjee *et al*., 2007). Thus, inhibitors of AChE activity (AChEIs) promote an increase in the concentration and duration of action of synaptic ACh (Rollinger *et al*., 2004). Currently, AChE inhibition becomes the remarkable alternative in treatment of AD (Martinez and Castro, 2006). AChEIs as eserine, tacrine, donepezil, rivastigmine and galanthamine are the only drugs now 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). Until now, no drug of choice for the treatment of this disease has been decided. Therefore, the search for new AChEIs is of great interest.

The history of drug discovery showed that plants are highly rich sources in the search for new active compounds and they have become a challenge to modern pharmaceutical industry. Many synthetic drugs take their origin from plant based on modern medicine. Since AD, one of the most common cause of death worldwide, has become a threaten to public health, new treatment strategies based on medicinal plants have been focused.

In this context, it is important to compare the activities of crude plant extracts, in order to provide a platform for further research on these plant species for the isolation of their active components. The present study was undertaken to evaluate the anticholinesterase potential of a number of selected Algerian medicinal plants with various ethnobotanical uses, aiming to discover new candidates for anticholinesterase compounds.

MATERIAL AND METHODS

Plant materials: Plant materials were collected in 2007 and 2008 at various locations in Algeria. The voucher

Corresponding Author: Abderrazak Marouf, Département de Biologie, Faculté des Sciences, Université d'Oran, BP 1524, 31000 Oran El-Menouar, Algeria Fax: +213(0) 41 41 60 21

specimens are kept at Faculty of Sciences, Department of Biology, Es-Senia University, Oran, Algeria. The plant materials were authenticated by one of us (Pr Abderrazak MAROUF). Botanical names and plant parts used to obtain the extracts are summarized in Table 1.

Preparation of plant extracts: The plant materials were cut into small pieces and dried in a hot air oven at 50°C. The aqueous extract was prepared by refluxing (60-70°C) 10 g of the plant material with 100 mL of distilled water for 30 min. This was repeated thrice with fresh solvent each time, followed by filtration. Filtered extracts were mixed and lyophilized to dryness.

Roots of *Cynomorium coccineum* L. and *Zizyphus lotus* L. were macerated in ethanol for 24 h and the mixture was filtered. The filtrate was evaporated under reduced pressure until dryness by rotary evaporator.

The methanolic extract was prepared by refluxing (60-70°C) 10 g of the plant material (*Ajuga iva* sp. *pseudo-iva* L. (aerial parts) and *Globularia alypum* L. (leaves)) with 100 mL of methanol for 30 min. This was repeated thrice with fresh solvent each time, followed by filtration. The filtrates were mixed and evaporated under reduced pressure until dryness by rotary evaporator.

Fractionation: The crude aqueous of *Atriplex halimus* L. roots extract (2.5 g) was suspended in distilled water (150 mL) and partitioned with chloroform (3×150 mL), ethyl acetate (3×150 mL) and n-butanol (3×150 mL) to yield the chloroform (14%), ethyl acetate (7.36%), n-butanol (22.56%) and aqueous (54%) fractions, respectively.

Chemicals: Acetylthiocholine Iodide (ATChI), Acetylcholinesterase (AChE), bovine serum albumin (BSA), 5, 5'-dithiobis[2-nitrobenzoic acid] (DTNB), α-naphtyl acetate and Fast blue B salt were obtained from Sigma (MO, USA). Gallic acid and catechin were obtained from Fluka Chemie (Buchs, Switzerland). All organic solvents (analytical-reagent grade) and TLC (DC-Alufolien, Silicagel 60 F_{254}, 0.2 mm thickness) were purchased from Merck (Darmstadt, Germany). AChE used in the assay was from electric eel (type VI-S lyophilized powder, 425.94 U mg^{-1} solid, 500 U mg^{-1} protein). The lyophilized enzyme was prepared in the buffer A (Tris–HCl, 50 mM, pH 8.0) to obtain 1000 U mL^{-1} stock solution and further diluted with buffer A containing 0.1% BSA to get 6.67 U mL^{-1}. DTNB (10 mM) was dissolved in the buffer B (NaH_2PO_4-Na_2HPO_4, 0.05 mM, pH 7.6). ATChI (200 mM) was dissolved in buffer A. α-naphtyl acetate (2.5 mg mL^{-1}) was dissolved in ethanol and Fast Blue B salt (2.5 mg mL^{-1}) was dissolved in distilled water.

TLC bioautography for acetylcholinesterase inhibition: Using Fast Blue B salt as reagent, the inhibitory activity of plant extracts was determined either with a TLC assay. The detailed procedure of the TLC assay has been described previously by Marston *et al*. (2002). Briefly, plant extracts dissolved in methanol, were spotted at 13.3 μg onto the TLC plates. Migration was conducted with AcOEt-HCOOH-AcOH-H_2O (100: 11: 11: 26; v/v). Then the plates were sprayed with an enzyme solution (30 mL, concentration 6.67 U mL^{-1}) and pre-incubated at 37 °C for 20 min in a humid atmosphere. Five mL of the α-naphthyl acetate solution (2.5 mg mL^{-1}) and 20 mL (2.5 mg mL^{-1}) of the Fast Blue B salt solution were mixed and sprayed onto the plates to give a purple coloration after 1-2 min. Plant extracts was noted for which an inhibition spot was still visible.

Spectrophotometric assay for AChE activity: AChE activity was measured using spectrophotometer (Double beam spectrophotometer Optizen, 2120 UV; used on visible range and operated on the kinetic mode. The absorbance data were acquired in a computer by means of Optizen view software 2001 (Mecasys Co. Ltd.), appropriate disposable plastic cuvettes (LP Italiana SPA, Italy, 2 mL) were used in the kinetic experiments) based on Ellman's method (Ellman *et al*., 1961). The enzyme hydrolyzes the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. In test tube, 1710 μL of buffer A, 250 μL of plant extracts in methanol (only plant extracts with AChE inhibition by TLC bioautography, extract of *Rosmarinus officinalis* L. and fractions of *A. halimus* L. roots) at various concentrations, 10 μL of 6.67 U mL^{-1} AChE and 20 μL of DTNB (10 mM) in buffer B were added. The mixture was incubated for 15 min at 37°C. Then, 10 μL of ATChI (200 mM) in buffer A were added to the mixture and the absorbance was measured at 412 nm every 10 sec for 3 min, for the blank sample 250 μL of buffer A was added instead of extract. The enzyme inhibition (%) was calculated from the rate of absorbance change with time (V = Abs/Δt) data as follows:

$$\text{Inhibition (\%)} = 100 - \frac{\text{Change of sample absorbance}}{\text{Change of blank absorbance}} \times 100$$

The experiment was done in triplicate.

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 versus the extract concentrations by using the Microsoft Excel program (2007).

Phytochemical screening: For each plant extracts a phytochemical screening was performed for the presence of secondary metabolites by using TLC analyses. The development was performed on aluminium plates coated with silica gel 60 F_{254}, 0.2 mm. The solvent systems are: AcOEt-MeOH-H_2O (100: 13.5: 10) (anthracenic derivatives, anthrones and anthranols), AcOEt-HCOOH-AcOH-H_2O (100: 11: 11: 26) (coumarins, flavonoids, phenolic acids, sesquiterpenes, lactons and triterpenes), AcOH (10%) (hydroxycoumarins), AcOEt-MeOH-H_2O (100: 13.5: 10) (cardiotonic glycosides), $CHCl_3$-MeOH-H_2O (70: 30: 4) (lignans), AcOEt-MeOH-H_2O (100: 17: 13) (free quinons), $CHCl_3$-MeOH-H_2O (64: 40: 8); (65: 35: 10) (saponins) and CH_2Cl_2-MeOH-NH_4OH (95: 5: 0.5) (alkaloids). The following spray reagents were used in order to develop the spots: anisaldehyde (triterpens), Folin-Ciocalteu (phenolic acids), KOH (anthracenic derivatives, anthrones and anthranols, coumarins and free quinons), Komarowsky (saponins), Neu (flavonoids), $SbCl_3$ (cardiotonic glycosids and lignans), Zimmermann (sesquiterpens lactons) and Dragendorff's reagent (alkaloids) (Fry, 1988; Wagner and Bladt, 1996).

Determination of total phenolic compounds: The total phenolic compound contents in the plant extracts were determined by colorimetric assay, using the Folin-Ciocalteu reagent (Heilerova *et al*., 2003) and gallic acid as a standard. Briefly, aliquot of 100 µL of the plant extracts in methanol was mixed with 500 µL of a 10% Folin-Ciocalteu reagent (in water) and the mixture was mixed thoroughly. After 5 min of incubation in obscurity, 1.5 mL of a 2% Na_2CO_3 (in water) was added; the mixture was mixed and incubated for 1h in obscurity. The absorbance was measured at 765 nm against blank prepared similarly, by replacing extract with methanol, using a spectrophotometer (Optizen, UV 2200). The concentration of total phenolic compounds, was determined as micrograms of acid gallic (4.76-23.8 µg mL^{-1}) equivalents by using an equation that was obtained from the standard gallic acid plots. Results were means of three assays expressed as gallic acid equivalents in mg g^{-1} of lyophilized plant extracts.

Determination of total flavonoids: The total flavonoid content was determined using the method described by Kim *et al.* (2003). An aliquot of 500 µL of plant extracts in methanol was mixed with 1500 µL of distilled water followed by addition of 150 µL of a 5% $NaNO_2$ (in water) and the mixture allowed to react for 5 min. Following this, 150 µL of a 10% $AlCl_3$ (in water) was added and the mixture stood for a further 6 min. Finally, the reaction mixture was treated with 500 µL of 1 M NaOH (in water) and the absorbance at 510 nm was obtained against blank prepared similarly, by replacing extract with methanol using a spectrophotometer (Optizen, UV 2200). Total flavonoid content was calculated from a calibration curve using catechin (5-25 µg mL^{-1}) as standard and expressed as mg of catechin equivalents g^{-1} of lyophilized plant extracts. The experiment was done in triplicate.

RESULTS AND DISCUSSION

Fifty three plant species were selected for investigation and a total of 77 extracts were tested for AChE inhibitory activity. The results obtained by TLC bioautography assay for acetylcholinesterase inhibition of all plant extracts are shown in Table 1.

Among the 77 extracts tested 11 showed AChE inhibition. TLC bioautography of active plants revealed active spots at different Rf values (Table 1). The active spots appeared as white spots on a purple background. Aqueous extracts from *A. raddiana* Savi (barks), *G. alypum* L. (roots), *O. quadripartita* Salzm. (leaves), *P. lentiscus* L. (leaves), *P. atlantica* Desf. (leaves) and *R. pentaphylla* L. (leaves) showed more than one spot for AChE inhibition. By means of TLC bioassay detection for AChE inhibition, aqueous extract from *P. lentiscus* L. (leaves) and *R. pentaphylla* L. (leaves) showed one spot of AChE inhibitors which was also positive with Neu reagent.

The inhibitory activities on AChE for the plant species examined were evaluated and the percentage inhibitions are shown in Table 2. It can be seen that among the 11 active extracts 4 showed potent AChE inhibition, 2 showed moderate inhibition and 5 showed low activities. For example, at the concentration of 20 µg mL^{-1}, the leaves extracts of *P. atlantica* Desf. and *P. lentiscus* L. showed high AChE inhibitory activity (with percent inhibition of 81.90±1.65% and 62.86±0.95%, respectively). At the concentration of 40 µg mL^{-1}, barks extract of *A. raddiana* Savi, showed 57.14±4.15% inhibitory activity on AChE. At the concentration of 50 µg mL^{-1}, the remaining of the extracts showed the AChE inhibitory activity below 51%.

The IC_{50} data of the aqueous extracts are given in Fig. 1. The strongest AChE inhibitory activities were exhibited by the aqueous extracts from leaves of *P. atlantica* Desf. (IC_{50} = 0.87±0.55), *P. lentiscus* L. (IC_{50} = 13.67±0.69 µg mL^{-1}), roots of *G. alypum* L. (IC_{50} = 16.67±6.13 µg mL^{-1}) and barks of *A. raddiana* Savi

Table 1: Screening of Algerian plants for acetylcholinesterase inhibitory activity by TLC bioautography assay

Family and species	Part used	Extraction solvent	Number of active constituents	Rf
Anacardiaceae				
Pistacia atlantica Desf.	Leaf	Water	2	0.38
				0.45
Pistacia lentiscus L.	Leaf	Water	7	0.03
				0.07
				0.28
				0.35
				0.62
				0.67
				0.76
Rhus pentaphylla L.	Leaf	Water	2	0
				0.52
	Fruit flesh	Water	0	-
	Seed	Water	0	-
Rhus tripartita Ucria	Leaf	Water	0	-
	Fruit	Water	0	-
	Leaf	Water	0	-
Apiaceae				
Ammodaucus leucotrichus Coss. and Dur.	Aerial part	Water	0	-
	Root	Water	0	-
Deverra scoparia L.	Aerial part	Water	0	-
Thapsia garganica L.	Leaf	Water	0	-
	Root (central part)	Water	0	-
	Root (bark)	Water	0	-
Aristolochiaceae				
Aristolochia baetica L.	Seed	Water	0	-
Asclepiadaceae				
Pergularia tomentosa L.	Leaf	Water	0	-
Solenostemma argel Del. Hayne	Leaf	Water	0	-
Asteraceae				
Atractylis humilis L.	Root	Water	0	-
	Leaf	Water	0	-
Perralderia coronopifolia Coss.	Flower	Water	0	-
	Foliar stem	Water	0	-
Scorzonera undulata Vahl.	Root	Water	0	-
Warionia saharae Benth. and Coss.	Leaf	Water	0	-
Berberidaceae				
Berberis vulgaris L.	Bark	Water	0	-
Borraginaceae				
Cynoglossum cheirifolium L.	Leaf	Water	0	-
Brassicaceae				
Zilla macroptera Coss.	Aerial part	Water	0	-
Caryophyllaceae				
Herniaria mauritanica Murb.	Aerial part	Water	0	-
Chenopodiaceae				
Atriplex halimus L.	Root	Water	1	0.05
	Leaf	Water	0	-
Fredolia aretioides Moq-Land. and Cof.	Leaf	Water	0	-
	Root	Water	0	-
Haloxylon scoparium Pomel.	Aerial part	Water	0	-
Cladoniaceae				
Cladonia rangiformis Hoffm.	Thallus	Water	0	-
Corallinaceae				
Coratlina officinalis L.	Thallus	Water	0	-
Cucurbitaceae				
Citrillus colocynthis Shrad.	Seed	Water	0	-
	Fruit flesh	Water	0	-
Cupressacea				
Juniperus phoenicea L.	Leaf	Water	0	-
Tetraclinis articulata (Vahl.) Mast.	Leaf	Water	1	0.05
Cynomoriaceae				
Cynomorium coccineum L.	Aerial part	Water	0	-
	Aerial part	Ethanol	0	-
Ephedraceae				
Ephedra altissima Desf.	Aerial part	Water	0	-
Ephedra major Host.	Aerial part	Water	0	-
Euphorbiaceae				
Euphorbia guyoniana Boiss. and Reuter.	Aerial part	Water	0	-

Table 1: Continued

Family and species	Part used	Extraction solvent	Number of active constituents	Rf
Fabaceae				
Acacia raddiana Savi	Bark	Water	2	0.83 0.77
	Leaf	Water	0	-
Astragalus gambo Coss. and Dur.	Aerial part	Water	0	-
Cassia aschrek Forssk.	Leaf	Water	0	-
Vigna radiata L. and R. Wilcz.	Leaf	Water	0	-
	Plantlet	Water	0	-
Gobulariaceae				
Globularia alypum L.	Flower	Water	0	-
	Root	Water	3	0.51 0.45 0.19
	Leaf	Water	0	-
	Leaf	Methanol	0	-
Lamiaceae				
Ajuga iva ssp. pseudo-iva L.	Aerial part	Water	0	-
	Aerial part	Methanol	0	-
Prasium majus L.	Leaf	Water	0	-
Rosmarinus officinalis L.	Leaf	Water	0	-
Moraceae				
Ficus ingens Miq.	Leaf	Water	0	-
Ficus salicifolia Vahl.	Leaf	Water	0	-
Myrtaceae				
Myrtus nivellei Batt and Trab.	Leaf	Water	1	0.7
Oleaceae				
Olea lapperini Batt and Trab.	Leaf	Water	1	0.59
Pinaceae				
Cedrus atlantica Endlicher. Manetti	Leaf	Water	0	-
Ramalinaceae				
Ramalina panizei De Not.	Thallus	Water	0	-
Rutaceae				
Ruta chalepensis L.	Leaf	Water	0	-
Salvadoraceae				
Salvadora persica L.	Leaf	Water	0	-
Santalaceae				
Osyris quadripartita Salzm.	Leaf	Water	2	0.3
	Immature fruit	Water	0	0.78
Solanaceae				
Solanum sodomaeum L.	Seed	Water	0	-
Withania frutescens L. and Pauquy	Leaf	Water	0	-
Thymeliaceae				
Thymelia hirsuta L. and Endl.	Aerial part	Water	0	-
Thymelia microphylla Coss. and Dur.	Foliar stem	Water	0	-
Zygophyllaceae				
Fagonia cretica L.	Aerial part	Water	0	-
Peganum harmala L.	Seed	Water	0	-
Zizyphus lotus Desf.	Seed	Water	0	-
	Fruit flesh	Water	0	-
	Root (bark)	Water	0	-
	Root (central part)	Ethanol	0	-

Table 2: Anticholinesterase activity of the plant extracts

	Inhibition (%)				
Plant species	AChE (5 μg mL^{-1})	AChE (10 μg mL^{-1})	AChE (15 μg mL^{-1})	AChE (20 μg mL^{-1})	AChE (25 μg mL^{-1})
P. atlantica	61.90±2.52	64.76±1.35	72.38±2.52	81.90±1.65	100±0
P. lentiscus	17.78±5.25	46.35±4.89	58.73±3.97	62.86±0.95	nd
Plant species	AChE (10 μg mL^{-1})	AChE (20 μg mL^{-1})	AChE (30 μg mL^{-1})	AChE (40 μg mL^{-1})	AChE (50 μg mL^{-1})
A. raddiana	24.76±0.95	38.41±4.89	47.62±1.65	57.14±4.15	64.13±1.10
Plant species	AChE (50 μg mL^{-1})	AChE (100 μg mL^{-1})	AChE (150 μg mL^{-1})	AChE (200 μg mL^{-1})	AChE (250 μg mL^{-1})
G. alypum	50.95±2.02	61.27±2.75	68.89±3.06	71.75±3.97	76.67±2.02
T. articulata	28.89±1.45	46.03±8.85	62.22±3.34	67.30±0.55	74.92±1.45
M. nivellei	21.90±3.30	43.49±5.74	61.27±4.50	78.57±2.02	85.71±2.52
O. lapperini	36.67±4.71	41.27±3.61	53.81±0.67	59.52±3.37	66.03±2.40
A. halimus	28.10±0.67	35.56±2.40	49.21±2.20	60.00±1.35	66.03±1.10
O. quadripartita	29.84±3.97	40.95±1.65	49.21±2.91	54.60±0.55	64.76±4.36
R. peutaphylla	11.90±3.37	29.21±4.40	38.10±0.95	57.46±2.91	67.30±3.06
R. officinalis	20.95±0.00	25.24±0.67	31.75±1.98	47.30±5.74	60.48±8.75

nd: Not determined. Values were expressed as Mean±SEM (n = 3)

Table 3: Acetylcholinesterase inhibition by the crude extract and subsequent fractions of *A. halimus* L.

	Inhibition (%)				
Fractions	AChE (25 μg mL^{-1})	AChE (50 μg mL^{-1})	AChE (75 μg mL^{-1})	AChE (100 μg mL^{-1})	AChE (125 μg mL^{-1})
$CHCl_3$	53.33±5.79	58.10±4.36	65.08±1.98	69.52±0.95	74.60±1.45
AcOEt	34.60±3.06	37.62±2.02	46.03±2.91	57.62±3.37	65.08±3.06
H_2O	14.60±3.85	22.54±1.45	37.78±1.10	47.62±2.52	56.67±2.02
n-BuOH	2.86±1.350	14.29±8.30	26.35±2.40	33.97±5.25	48.10±0.67
	Inhibition (%)				
Crude extract	AChE (50 μg mL^{-1})	AChE (100 μg mL^{-1})	AChE (150 μg mL^{-1})	AChE (200 μg mL^{-1})	AChE (250 μg mL^{-1})
A. halimus	28.10±0.67	35.56±2.40	49.21±2.20	60.00±1.35	66.03±1.10

Values were expressed as Mean±SEM (n = 3)

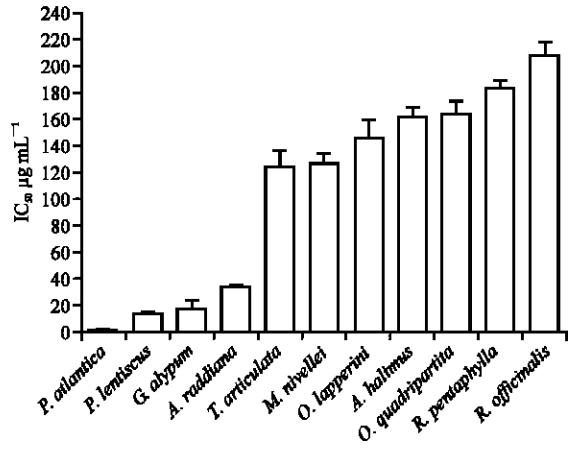

Fig. 1: IC_{50} values for acetylcholinesterase inhibition by plant extracts. Data are given as Mean±SEM (n = 3)

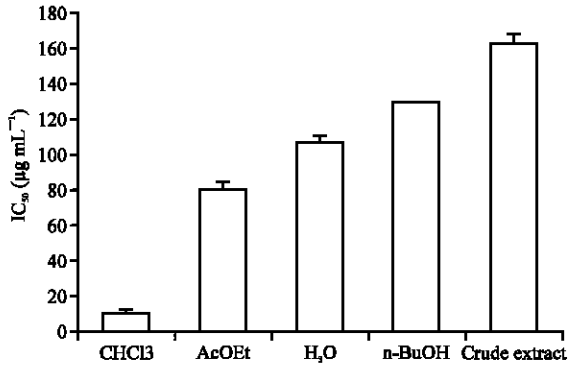

Fig. 2: IC_{50} values for acetylcholinesterase inhibition by crude extract and fractions of *A. halimus* L. Data are given as Mean±SEM (n = 3)

IC_{50} = 33.91±0.94 μg mL^{-1}). Currently no AChE inhibitory activity has been reported from these plants. The water extract from leaves of *Rosmarinus officinalis* L., exhibits the lowest AChE inhibition with IC_{50} = 207.41±10.04 μg mL^{-1}.

The results obtained with the crude extract and subsequent fractions of *A. halimus* L., for inhibitory activity against acetylcholinesterase are shown in Table 3 and Fig. 2. The chloroform fraction gives the highest inhibition (IC_{50} = 9.55±2.91 μg mL^{-1}), percentage of inhibition was 74.60±1.45% at 125 μg mL^{-1}.

The phytochemical tests carried out on the active aqueous extracts made it possible to highlight the various phytochemical groups present in the plants, recapitulated in Table 4.

The amount of total phenolics varied in different plants and ranged from 438.99±7.52 to 18.67±2.22 mg GA g^{-1} of extract. The extract of *O. quadripartita* Salzm. leaves was found to contain the highest total polyphenol content among the extracts tested (438.99±7.52 mg g^{-1}), followed by *P. atlantica* Desf. (407.68±9.23 mg g^{-1}) and *P. lentiscus* L. leaves (361.59±11.07 mg g^{-1}).

Using the $AlCl_3$ reagent and quercetin as standard (R^2 = 0.97), the total flavonoids varied from 125.70±11.3 to 9.85±1.93 mg CT g^{-1} for aqueous extracts.

TLC bioassay is an easier and rapid means for detection of enzyme inhibition. Furthermore, it permits to localize directly the compound responsible for this inhibition, within the all constituents of a crude extract, without further fractionation. The aqueous extract from *P. lentiscus* L. (leaves) and *R. pentaphylla* L. (leaves) showed one spot of AChE inhibitors which was also positive with Neu reagent. This result suggests that the AChE inhibitors present in these species extracts may be flavonoids. The result is consistent with the finding of Barbosa-Filho *et al.* (2006) on the AChE inhibitory activity of flavonoids from plant extracts. *P. lentiscus* L. (leaves) is known to contain a number of flavonoids: catechin, quercetin, myricetin (Romani *et al.*, 2002; Vaya and Mahmood, 2006). However, quercetin is identified as a potent AChE inhibitor (Jung and Park, 2007).

The species of *Pistacia* tested exhibit AChE inhibition highest than *Semecarpus anacardium* Linn.f. (16.74 μg mL^{-1}) plant from same family (Anacardiaceae)

Table 4: Phytochemical screening of plant extracts

Phytoconstituents	*A. raddiana*	*A. halimus*	*G. alypum*	*M. nivellei*	*O. lapperini*	*O. quadripartita*
Phenolic acids	cinnamic acid	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Anthrones and anthranols	-	-	-	-	-	+
Coumarins	-	-	-	-	-	-
Hydroxycoumarins	+++	-	+	-	-	++
Anthracenic derivatives	-	-	-	-	-	-
Flavonoids	-	+	++	+	++	+
Cardiotonic glycosids	+	-	+	+	+	-
Lignans	-	-	+	nd	nd	+
Free quinons	-	-	-	nd	nd	-
Saponins	-	+	+	-	+	-
Sesquiterpene lactons	+	-	-	-	-	+
Triterpenes	-	-	-	+	+	-

Phytoconstituents	*P. Atlantica*	*P. lentiscus*	*R. pentaphylla*	*R. officinalis*	*T. articulata*
Phenolic acids	-	-	-	-	-
Alkaloids	-	-	-	-	-
Anthrones and anthranols	-	-	+	-	-
Coumarins	-	-	-	-	-
Hydroxycoumarins	-	++	++	-	-
Anthracenic derivatives	-	-	-	-	-
Flavonoids	+++	+++	+++	-	-
Cardiotonic glycosids	-	-	-	+	-
Lignans	nd	-	-	nd	-
Free quinons	nd	-	-	nd	-
Saponins	-	-	-	-	-
Sesquiterpene lactons	-	++	-	-	-
Triterpenes	-	-	-	+	-

-: Absent; +: Low quantity; ++: High quantity; +++: Very high quantity, nd: Not determined

(Vinutha *et al*., 2007). The activity also has been found in three other species of *Acacia*, i.e., *Acacia seyal* Del., *A. nilotica* L. Willd. ex Del. sp. *kraussiana* and *A. sieberiana* Dc. var. *woodii* (Burtt Davy) (Eldeen and van Staden, 2007).

The lowest AChE inhibition (IC_{50} = 207.41±10.04 μg mL^{-1}) is exhibited by the water extract from leaves of *Rosmarinus officinalis* L. This inhibition is more active than as identified by Mata *et al*. (2007) for aqueous extract from rosemary leaves (IC_{50} = 769±3.9 μg mL^{-1}). Several monoterpenes are known to inhibit AChE (Howes *et al*., 2003). Rosemary is aromatic plant, known to contain various monoterpenes. The AChE inhibition activity observed for the potent extracts partly offers evidence for use of these herbs in traditional medicine to enhance cognition or to correct cognitive decline.

Terrestrial plants offer a unique and renewable resource in order to discover potential new drugs and biological entities, because of the structural and biological diversity of their constituents. At the same time, global interest is increasing in natural products because of safety concerns for synthetic products (Gijtenbeek *et al*., 1999). In industrialized nations at the present time, some 50% of all prescribed drugs are derived or synthesized from natural products. The WHO has estimated that for some 3.4 billions peoples in the developing world, plants represent the primary source of medicine. *In vitro* inhibitory activity of the crude aqueous extract and subsequent fractions of *A. halimus* L. roots were carried out against acetylcholinesterase to probe potential AChE inhibition activity.

As shown in Fig. 2, the chloroform fraction gives the highest inhibition (IC_{50} = 9.55±2.91 μg mL^{-1}), percentage of inhibition was 74.60±1.45% at 125 μg mL^{-1}. It is seen also that the acetylcholinesterase inhibition of the other fractions is decreasing in the following order: ethyl acetate > water > butanol > crude extract. It can be postulated that the crude extract and subsequent fractions of *A. halimus* L. have a strong potential to inhibit the AChE and can, therefore, offer a great opportunity for the isolation of active agents for the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson disease and myasthenia gravis. Thus, these findings also provide a platform for further research on this plant species.

The TLC phytochemical screening carried out on the active aqueous extracts (Table 4) allowed the identification of a pattern of anthrones and anthranols from *O. quadripartita* Salzm. and *R. pentaphylla* L., cardiotonic glycosids from *A. raddiana* Savi, *G. alypum* L., *M. nivellei* Batt and Trab., *O. lapperini* Batt and Trab. and *R. officinalis* L. hydroxycoumarins from *A. raddiana* Savi, *O. quadripartita* Salzm., *P. lentiscus* L., *R. pentaphylla* L. and *G. alypum* L. flavonoids from *P. atlantica* Desf., *P. lentiscus* L., *R. pentapylla* L., *G. alypum* L., *O. lapperini* Batt and Trab., *A. halimus* L.,

M. nivellei Batt and Trab., *O. quadripartita* Salzm.; lignans from *G. alypum* L. and *O. quadripartita* Salzm.; phenolic acids (cinnamate) from *A. raddiana* Savi; saponins from *A. halimus* L., *G. alypum* L. and *O. lapperini* Batt and Trab.; sesquiterpene lactons from *P. lentiscus* L., *A. raddiana* Savi and *O. quadripartita* Salzm.; and triterpenes from *M. nivellei* Batt and Trab., *O. lapperini* Batt and Trab. and *R. officinalis* L. Alkaloids, anthracenic derivatives, free quinons and coumarins have not been detected for all plants tested. However, several triterpenes have been isolated from leaves of rosemary such as ursolic, oleanolic and betulinic acids (Islamcevic-Razborsek *et al*., 2008). Different flavonoids were described for *P. lentiscus* L. myricetin, quercitin glycoside, catechin and genistein (Vaya and Mahmood, 2006). It has been shown that the Anacardiaceae family is characterized by the occurrence of myricetin derivatives (Umadevi *et al*., 1988). The abundance of the flavonoids glycosides was also noted in the aerial parts of *P. lentiscus* L. and *P. atlantica* Desf. (Kawashty *et al*., 2000). Es-Safi *et al*. (2005) have demonstrated that the aerial parts of *G. alypum* L. contain flavonoid glycosides such as 6-hydroxyluteoline7-*O*-β-D-glucopyranoside and luteoline 7-*O*-sophoroside. Moreover, two lignan glycosides were isolated from *G. alypum* L.: syringaresinol 4'-O-β-D-glucopyranosid and liriodendrin (Chaudhuri and Sticher, 2004; Kirmizibekmez *et al*., 2007).

The *O. quadripartita* Salzm. leaves extract was found to contain the highest total phenolics content among the extracts tested (438.99±7.52 mg g^{-1}), followed by *P. atlantica* Desf. (407.68±9.23 mg g^{-1}) and *P. lentiscus* L. leaves (361.59±11.07 mg g^{-1}). The value obtained for the aqueous extracts of *P. atlantica* Desf. and *P. lentiscus* L. is greatly above that reported in the literature for an specie belong to the same family *Comocladia egleriana* Loes (215 mg g^{-1}) (Ruiz-Terαn *et al*., 2008). The value found for the aqueous extract of rosemary, (168.18±12.53 mg g^{-1}) is in the same order of that reported in the literature, (162 mg g^{-1}) (Erkan *et al*., 2008).

The total flavonoids varied from 125.70±11.3 to 9.85±1.93 mg CT g^{-1} for aqueous extracts. The highest value was 125.70±11.33 mg CT g^{-1} (*R. officinalis* L.) and 115.37±11.28 mg CT g^{-1} (*A. raddiana* Savi), the lowest was 9.85±1.93 mg CT g^{-1} (*A. halimus* L.) with the following decreasing order: *R. officinalis* L. > *A. raddiana* Savi > *O. laperini* Batt and Trab. > *O. quadripartita* Salzm. > *G. alypum* L. > *P. atlantica* Desf. > *P. lentiscus* L. > *T. articulata* Vahl. Master > *R. pentaphylla* L. > *M. nivelei* Batt and Trab.> *A. halimus* L. The concentration of total flavonoids in the tested *R. officinalis* L. was higher than in the other Lamiaceae plants such as *Teucrium polium* L., *T. chamaedrys* L. and *T. montanum* L. (Panovska *et al*., 2005). The value obtained for the aqueous extract of *A. raddiana* Savi barks is twice superior than that reported in the literature for a species belong to the same family: *Albizia amara* Roxb. Boivin (Kumar *et al*., 2008). We also mention here, that an increase of the total flavonoids for this specie may be related to the hard climate conditions (hot temperatures, high solar exposure, dryness, short growing season).

CONCLUSIONS

Among the 53 Algerian plants analyzed several showed inhibitory activity of the enzyme acetylcholinesterase. *P. Atlantica* Desf., *P. lentiscus* L., *G. alypum* L. and *A. raddiana* Savi, presented good IC_{50} values for inhibition of acetylcholinesterase. The aqueous extract of *P. atlantica* Desf. showed the best anticholinesterase activity. *P. atlantica* Desf. used as medicinal plants, may help in preventing or alleviating patients suffering from Alzheimer's disease. Finally, plants showing AChE inhibitory capacity could be considered as candidates for further studies in the treatment of Alzheimer's disease.

Further investigations on the AChE inhibiting extracts (isolation, purification and structural determination of the active constituents through bioassay-directed fractionation) could lead to candidate compounds helpful in diseases like AD. Others major perspectives may be focalized on the determination of AChE inhibition mode of the active substances and the study of structure-activity relation.

ACKNOWLEDGMENT

The authors thank the Agence Nationale pour le Développement de la Recherche en Santé, (ANDRS, Algeria) for financing of the acquisition of reagents.

REFERENCES

Barbosa-Filho, J.M., K.C.P. Medeiros, M.F.F.M. Diniz, L.M. Batista and P.M. Athayde-Filho *et al*., 2006. Natural products inhibitors of the enzyme acetylcholinesterase. Rev. Bras. Farmacogn., 16: 258-285.

Chaudhuri, R.K. and O. Sticher, 2004. New iridoid glucosides and a lignan diglucoside from *Globularia alypum* L. Helv. Chim. Acta, 64: 3-15.

Eldeen, I.M.S. and J. van Staden, 2007. *In vitro* pharmacological investigation of extracts from some trees used in *Sudanese traditional* medicine. S. Afr. J. Bot., 73: 435-440.

Ellman, L., K.D. Courtney, V. Andresjr and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Erkan, N., G. Ayranci and E. Ayranci, 2008. Antioxidant activities of rosemary (*Rosmarinus officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem., 110: 76-82.

Es-Safi, N.E., S, Khlifi, L, Kerhoas, A, Kollmann, A, El-Abbouyi and P.H. Ducrot, 2005. Antioxidant constituents of the aerial parts of *Globularia alypum* growing in Morocco. J. Nat. Prod., 68: 1293-1296.

Fry, S.C., 1988. The Growing Plant Cell Wall: Chemical and Metabolic Analysis. Longman Scientific and Technical, Harlow, Essex, UK., ISBN: 0470210796, pp: 333.

Gijtenbeek, J.M.M., M.J. van den Bent and C.J. Vecht, 1999. Cyclosporine neurotoxicity: A review. J. Neurol., 246: 339-346.

Heilerova, L., M. Buckova, P. Tarapcik, S. Silhar and J. Labuda, 2003. Comparison of antioxidative activity data for aqueous extracts of Lemon Blam (*Melissa officinalis* L.), Oregano (*Origanum vulgare* L.), Thyme (*Thymus vulgaris* L.) and Agrimony (*Agrimonia eupatoria* L.) obtained by conventional method and the DNA-based biosensor. Czech J. Food Sci., 21: 78-84.

Howes, M.J.R., N.S.L. Perry and P.J. Houghton, 2003. Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother. Res., 17: 1-18.

Islamcevic-Razborsek, M., D. Brodnjak-Voncina, V. Dolecek and E. Voncina, 2008. Determination of oleanolic, betulinic and ursolic acid in Lamiaceae and mass spectral fragmentation of their trimethylsilylated derivatives. Chromatographia, 67: 433-440.

Jung, M. and M. Park, 2007. Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. Molecules, 12: 2130-2139.

Kırmızıbekmez, H., S. Piacente, C. Bassarello and I. Çalış, 2007. New phenylethanoid glycosides from *Globularia alypum*. Planta Med., 73: 889-890.

Kawashty, S.A., S.A.M. Mosharrafa, M. El-Gibali and N.A.M. Saleh, 2000. The flavonoids of four *Pistacia* species in Egypt. Biochem. Syst. Ecol., 28: 915-917.

Kim, D., S.W. Jeong and C.Y. Lee, 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. J. Agric. Food Chem., 81: 321-326.

Kumar, P.S., S. Sucheta, V.S. Deepa, P. Selvamani and S. Latha, 2008. Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol., 7: 1826-1828.

Lahiri, D.K., M.R. Farlow, N.H. Greig and K. Sambamurti, 2002. Current drug targets for Alzheimer's disease treatment. Drug Dev. Res., 56: 267-281.

Marston, A., J. Kissling and K. Hostettmann, 2002. A rapid TLC bioautographic method for the detection of acetylcholinesterase and butyrylcholinesterase inhibitors in plants. Phytochem. Anal., 13: 51-54.

Martinez, A. and A. Castro, 2006. Novel cholinesterase inhibitors as future effective drugs for the treatment of Alzheimer's disease. Expert Opin. Invest. Drugs, 15: 1-12.

Mata, A.T., C. Proença, A.R. Ferreira, M.L.M. Serralheiro, J.M.F. Nogueira and M.E.M. Araújo, 2007. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. Food Chem., 103: 778-786.

Mukherjee, P.K., V. Kumar, M. Mal and P.J. Houghton, 2007. Acetylcholinesterase inhibitors from plants. Phytomedicine, 14: 289-300.

Panovska, T.K., S. Kulevanova and M. Stefova, 2005. *In vitro* antioxidant activity of some *Teucrium* species (*Lamiaceae*). Acta. Pharm., 55: 207-214.

Rollinger, J.M., A. Hornick, T. Langer, H. Stuppner and H. Prast, 2004. Acetylcholinesterase inhibitory activity of scopolin and scopoletin discovered by virtual screening of natural products. J. Med. Chem., 47: 6248-6254.

Romani, A., P. Pinelli, C. Galardi, N. Mulinacci and M. Tattini, 2002. Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of *Pistacia lentiscus* L. Phytochem. Anal., 13: 79-86.

Ruiz-Teran, F., A. Medrano-Martínez and A. Navarro-Ocaña, 2008. Antioxidant and free radical scavenging activities of plant extracts used in traditional medicine in Mexico. Afr. J. Biotechnol., 7: 1886-1893.

Sung, S.Y., S.Y. Kang, K.Y. Lee, M.J. Park and J.H. Kim *et al*., 2002. (+)-α-Viniferin, a stilbene *Trimer* from *Caranga chamlague* inhibits acetylcholinesterase. Biol. Pharm. Bull., 25: 125-127.

Umadevi, I., M. Daniel and S.D. Sabnis, 1988. Chemotaxonomic studies on some members of *Anacardiaceae*. Proc. Indian. Acad. Sci., 98: 205-208.

Vaya, J. and S. Mahmood, 2006. Flavonoid content in leaf extracts of the fig (*Ficus carica* L.), carob (*Ceratonia siliqua* L.) and pistachio (*Pistacia lentiscus* L.). Biofactors, 28: 169-175.

Vinutha, B., D. Prashanth, K. Salma, S.L. Sreeja and D. Pratiti *et al*., 2007. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. J. Ethnopharmacol., 109: 359-363.

Wagner, H. and S. Bladt, 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd Edn., Springer Verlag, Heidelberg.