Journal of Enzyme Inhibition and Medicinal Chemistry, June 2009; 24(3): 818–824

informa
healthcare

A potential role of alkaloid extracts from *Salsola* species (Chenopodiaceae) in the treatment of Alzheimer's disease

ROSA TUNDIS, FEDERICA MENICHINI, FILOMENA CONFORTI, MONICA R. LOIZZO, MARCO BONESI, GIANCARLO STATTI, & FRANCESCO MENICHINI

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Nutritional and Health Sciences, University of Calabria, I-87030 Rende (CS), Italy

(*Received 7 May 2008; accepted 7 July 2008*)

Abstract

From the aerial parts of *Salsola oppositofolia*, *S. soda* and *S. tragus* an alkaloid extract was obtained and tested to evaluate antioxidant and anti-cholinesterase activities. The *in vitro* study of the antioxidant activity by the DPPH method revealed a significant activity of *Salsola* alkaloid extracts with IC_{50} values ranging from 16.30 μg/mL for *S. oppositifolia* to 26.17 μg/mL for *S. tragus*. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities were evaluated. *S. tragus* alkaloid extract exerted the highest inhibitory activity against AChE (IC_{50} of 30.2 μg/mL) and BChE (IC_{50} of 26.5 μg/mL). Interestingly, *S. soda* and *S. oppositifolia* exhibited a selective inhibitory activity against BChE with IC_{50} values of 34.3 μg/mL and 32.7 μg/mL, respectively. Tetrahydroisoquinoline alkaloids were identified and quantified by GC/MS analysis.

Keywords: Salsola *species, tetrahydroisoquinoline alkaloids, acetylcholinesterase, butyrylcholinesterase, inhibition, antioxidant activity*

Introduction

In spite of the great advances in modern medicine in recent decades, plants still make an important contribution to health care. Plants provide chemical diversity and bioactivity, which has led to the development of hundreds of pharmaceutical drugs. Natural products represent an unparalleled source of molecular diversity for drug discovery and development. Oxidative stress can result from an increase in the levels of reactive oxygen species (ROS) that are produced as a result of many biochemical reactions and are considered to be the prime causes of oxidative damage, including protein denaturation, mutagenesis and lipid peroxidation in aerobic cells. Among these free radicals, the hydroxyl radical is one of the most aggressive found in living beings, reacting at a controlled diffusion rate with molecules such as DNA, lipids, proteins and carbohydrates. Oxidative damage, caused by the action of free radicals, may initiate and promote the progression of a number of chronic diseases, including neurodegenerative diseases, cancer and diabetes [1–3]. Alzheimer's disease (AD) is one of the most common neurodegenerative diseases. In spite of the multi-factorial nature of AD, only one therapeutic approach was currently followed. This strategy is based on the so-called cholinergic hypothesis of cognitive dysfunction [4]. This hypothesis postulates that at least some of the cognitive decline experienced by patients of AD results from a deficiency in neurotransmitter acetylcholine (ACh) and thus in cholinergic neurotransmission, which seems to play a fundamental role in memory [5]. On the one hand, cholinergic neurons are markedly damaged in AD patient brains, together with a defect in choline acetyltransferase and acetylcholinesterase (AChE) [6]. Moreover, in late stage of AD, levels of AChE declines by up to 85% and BChE represents the predominant cholinesterase in the brain. Butyrylcholine (BchE), primarily associated with glial cells, but also with specific neuronal pathways, cleaves ACh in a manner similar

Correspondence: R. Tundis, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Nutritional and Health Sciences, University of Calabria, I-87030 Rende (CS), Italy. Tel: 39 984 493246. Fax: 39 984 493298. E-mail: tundis@unical.it

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360802399662

to AChE to terminate its physiological action [7–9]. Such studies, together with a statistically slower decline in the cognitive performance of dementia patients possessing specific BChE polymorphisms that naturally lower BChE activity, have targeted BChE as a new approach to intercede in the progression of AD [10,11]. Currently, cholinesterase inhibition is the most used treatment for the symptoms of AD and AChE as well as BChE are therapeutic targets for improving the cholinergic deficit [12]. The potential use of a large number of naturally-occurring compounds has been successfully demonstrated in the field of AD. Majority of studies have focused on the anti-cholinesterase alkaloids, such as physostigmine and galantamine. So far, more than 35 alkaloids have been reported to have AChE inhibitory activity. The other major classes of compound reported to have such activity are the terpenoids, glycosides and coumarins [13].

As a part of our efforts to find new antioxidative and cholinesterase active compounds we have investigated alkaloid extracts of *Salsola* species, such as *S. tragus*, *S. soda* and *S. oppositifolia*. The genus *Salsola* (Chenopodiaceae) comprises about 120 species; they are widespread herbaceous or shrubby plants, especially in the brackish grounds of the moderate and subtropical regions of Europe, Asia, Africa and North America. *Salsola* species are well known in folk medicine of Russia, China and Bahrain where there used such as diuretic, anti-hypertensive, anti-cancer, purgative, emollient, anti-ulcer and anti-inflammatory [14–16]. Some *Salsola* species, such as *S. kali* and *S. soda*, were used as food. *Salsola soda* is still cultivated as a vegetable that enjoys considerable popularity in Italy and with gourmets around the world. Its common names in Italian include "Barba di Frate", "Agretti roscana" and "Liscari sativa". Our previously studies supported scientifically the traditional use of *Salsola* species in the treatment of hypertension and demonstrated the hypoglycaemic effects of *S. soda*, *S. tragus* and *S. oppositifolia* extracts by the inhibition of α-amylase enzyme [17,18]. Phytochemical evaluations of *Salsola* species resulted in the isolation of flavonoids, acetophenones, coumarines and sterols [19–22]. Simple tetrahydroisoquinoline alkaloids are also common in the Chenopodiaceae family and were isolated in different *Salsola* species [23–25]. In the last years, the attention of a number of researchers focused on the study of both biochemical and pharmacological properties of tetrahydroisoquinoline alkaloids (TIQs). The tetrahydroisoquinoline alkaloids affect the vegetative nervous system [26]. Simple isoquinoline alkaloids display potent and often selective cytotoxicity or exhibit potential antimicrobial, antimalarial, antiviral and anti-HIV activities [27]. This class of natural compound was reported in literature to possess hypothensive activity through the Ca^{2+} antagonistic properties. Recently, some (1,3′)-bis-tetrahydroisoquinolines were reported as scaffold intermediates for the synthesis of pentacyclic piperazine core alkaloids and their cytotoxicity against cancerous cell lines was evaluated [28]. Several isoquinoline alkaloids showed interesting cholinesterase inhibitory activity [29–31]. Among them, (-)-galanthamine, a natural alkaloid belonging to the Amaryllidaceae family, is a selective, long-acting, reversible and competitive acetylcholinesterase inhibitor that has been approved for the symptomatic treatment of AD. On the basis of these considerations we decided of tested antioxidant and anti-cholinesterase activities of *Salsola* species alkaloid extracts for establish a potential role in the treatment of Alzheimer's disease.

Materials and methods

General

Methanol, ethanol, dichloromethane, ethyl acetate, sulphuric acid, NH_4OH, Dragendorff's reagent (DRG) and anhydrous sodium sulphate were purchased from VWR International (Milan, Italy). Acetylcholinesterase (AChE) from *Electrophorus electricus* (EC 3.1.1.7, Type VI-S) and butyrylcholinesterase (BChE) from equine serum (EC 3.1.1.8), physostigmine, 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and salsolinol hydrochloride were purchased from Sigma-Aldrich (Milan, Italy). TLC analyses were carried out on silica gel 60 F_{254} precoated plates (VWR International, Milan, Italy).

Plant material

The aerial parts of *S. tragus* L. (voucher specimen n. 12344) and *S. oppositifolia* Desf. (voucher specimen n. 12345) were harvested in Southern Italy (Calabria and Sicily, respectively), while the aerial parts of *S. soda* L. (voucher specimen n. 12346) were harvested in Central Italy (Tuscany). Plant materials were collected and identified by Dr. N.G. Passalacqua of the Natural History Museum of Calabria and Botanical Garden of Calabria University and Dr. L. Peruzzi Biology Department, University of Pisa. Voucher specimens were deposited in the Botany Department Herbarium at the Calabria University (CLU), Italy.

Extracts preparation

Air-dried aerial parts (400 g) of each species were powdered and exhaustively extracted with methanol for 48 h (3 × 2.5 L) at room temperature. Combined methanol solutions were concentrated under reduced pressure and dried then suspended in distilled water, alkalized with NH_4OH (up to pH 8) and extracted by ethyl acetate no more alkaloids could be detected in aqueous phase. The organic portions were dried over anhydrous sodium sulphate and the solvent evaporated to dryness to obtain the alkaloid extracts. In order to evaluate the presence of alkaloids thin layer

chromatography (TLC) of methanol and ethyl acetate extracts of the three *Salsola* species was performed on silica gel F254 VWR (Milan, Italy). Mobile phase was a 85:14:1 mixture of CH_2Cl_2/MeOH/NH_4OH. TLC were analyzed by UV (254 and 365 nm) and were sprayed with Dragendorff's reagent.

Gas chromatography/mass spectrometry (GC/MS) analyses

GC/MS analyses of *Salsola* species alkaloid extracts were carried out using a Hewlett-Packard 6890 gas chromatograph equipped with an SE-30 capillary column (30 m length, 0.25 mm i.d., 0.25 μ film thickness) and interfaced with a Hewlett Packard 5973 Mass Selective. Ionization of the sample components was performed in electron impact mode (EI, 70 eV). The carrier gas was helium (1 mL/min) and the analytical conditions worked with the following program: oven temperature was 5 min isothermal at 50°C, then 50-250°C at a rate of 5°C/min; then held isothermal for 10 min. Injector and detector were maintained at 250°C and 280°C, respectively. For analysis, extracts were dissolved in dichloromethane and aliquots (1 μL) were directly injected. For quantitative determinations, an internal standard method was used to compare peak areas with the amounts of standard (the results given are the averages of three determinations each).

Antioxidant activity (DPPH Assay)

Free radical scavenging activity was determined using a rapid TLC screening method based on the reduction of a methanolic solution of the coloured free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). After developing and drying TLC plates were sprayed with a 0.2% DPPH solution in MeOH. The plates containing the essential oils were examined 30 min after spraying. The samples with antioxidant activity appeared as yellow spots against a purple background. In order to determine the radical scavenging potency the samples, which exhibited antioxidant activity, were investigated with an experimental procedure that was adapted from Wang et al. 1998 [32]. In an ethanol solution of DPPH radical (final concentration was 1.0×10^{-4} M), test samples were added at different concentrations. The reaction mixtures were shaken vigorously and then kept in the dark for 30 min. The absorbance of the resulting solutions was measured in 1 cm cuvettes using a Perkin Elmer Lambda 40 UV/VIS spectrophotometer at 517 nm against blank, which was without DPPH. All tests were run in triplicate and averaged. Ascorbic acid was used as a positive control.

Microtitre Cholinesterase inhibition assay

Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by slightly modifying the spectrophotometric method previously developed by Ellman et al. [33], which is based on the reaction of released thiocoline to give a colored product with chromogenic reagent *Electrophorus electricus* (EC 3.1.1.7, Type VI-S) AChE and equine serum (EC 3.1.1.8) BChE were used, while acetylthiocholine iodide and butyrylthiocholine iodide, respectively, were used as substrates of the reaction. The 5,5′-dithiobis(2-nitrobenzoic-acid) (DTNB) was used for the measurement of the cholinesterase activity. In this procedure, AChE or BChE (0.20 U/mL in buffer pH 8) and *Salsola* extracts at final concentrations in the test solution ranging from 20 to 500 μg/ml (20 μL) were added to 2 mL of buffer pH 8 and pre-incubated in ice bath at 4°C for 30 min. Test extracts and control were dissolved in 5% MeOH. Duplicate tubes were also treated this way with 20 μL of physostigmine (0.1 mM) to allow interference of the test substances in the assay to be assessed, and to control for any hydrolysis of acetylcholine or butyrylcholine not due to enzyme activity. The reaction was started by adding DNTB solution (20 μL of 0.05 mM in buffer pH 7) and acetylthiocholine iodide (ATCI) or butyrylthiocholine iodide (BTCI) (20 μL 0.018 mM in buffer pH 7) and tubes were allow in water bath for 20 min at 37°C. The reaction was halted by placing the assay solution tubes in an ice bath and adding physostigmine (20 μL 0.018 mM in buffer pH 7). Blanks were used of reagents without extracts and the positive control physostigmine (20 μL 0.018 mM in buffer pH 7) was added. The hydrolysis of acetylthiocholine and butyrylthiocholine was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion, as the result of the reaction of DTNB with thiocholine, released during enzymatic hydrolysis, immediately recorded on spectrophotometer (Jenway 6300) at 405 nm and the percentage inhibition was calculated. The samples and the standard (physostigmine) was dissolved in 5% methanol which was used for the control. All the reactions were performed in triplicate. The inhibition rate (%) was calculate by equation:

$$\text{Inhibition } \% = \frac{[(\text{Blank} - \text{Blank positive control}) - (\text{Experiment} - \text{Experiment control})]}{(\text{Blank} - \text{Blank positive control})}$$

Statistical analysis

Differences were evaluated by one-way analysis of variance (ANOVA) test completed by a multicomparison Dunnett's test. Differences were considered significant at $^{**}p < 0.01$. The inhibitory concentration 50% (IC_{50}) was calculated from dose-response curve obtained by plotting the percentage of inhibition versus the concentrations with the use of GraphPad Prism 4.0 Software.

Results and discussion

Alkaloids content

By maceration of the aerial parts of three *Salsola* species a methanolic extract at a yield of 8.51%, 7.61% and 10.81% (relative to dry weight material) was obtained for *S. oppositifolia*, *S. soda* and *S. tragus*, respectively. By partition with ethyl acetate in alkaline media, an alkaloid extract was obtained. The yields for *S. oppositifolia*, *S. soda* and *S. tragus* were 0.17%, 0.03% and 0.11%, respectively. GC/MS represents a powerful high-resolution method for the identification of underivatized alkaloids from biological sources. The results of GC/MS analyses and the contents of tetrahydroisoquinoline alkaloids identified in *Salsola* species are summarized in Tables I and II. The alkaloid contents were given as w/w % of dried alkaloid extract. The GC/MS investigation resulted in the identification of four main tetrahydroisoquinoline alkaloids by their MS fragmentation patterns as major components of the ethyl acetate extracts (Figure 1). Identification was made also by comparison of their spectral fragmentation with those reported in the literature [34]. In relation to the mass fragmentation pattern, the ions at *m/z* 192, 178 and 149 are characteristic. Salsoline and salsolidine were the major alkaloids in all studied *Salsola* species. Particularly, the amounts of salsoline and salsolidine were significantly higher in *S. tragus* with percentage of 36.5% and 17.7%, respectively. Interestingly, *S. oppositifolia* alkaloid extract showed the most high content of *N*-methylisosalsoline (6.3%). The change in alkaloids content of the three *Salsola* species influenced the anticholinesterase activity.

Table I. MS data of tetrahydroisoquinoline alkaloids in *Salsola* species.

Alkaloid	$[M]^+$ (EI-MS)	*m/z* (abundance %)
Salsoline	193	192 (12), 178 (100), 163 (17), 149 (11), 134 (7), 122 (6)
Salsolidine	207	192 (100), 190 (60), 178 (55), 177 (45), 163 (30), 149 (8)
N-Methylisosalsoline	207	192 (100), 177 (45), 164 (10), 149 (15), 121 (5), 96 (6)
Carnegine	221	220 (2), 206 (100), 207 (14), 190 (10), 178 (2), 145 (2)

DPPH radical scavenging activity

The antioxidant activity of the different *Salsola* species was carried out using DPPH test. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidant activities in a relatively short time. The absorbance decreases as a result of a color change from purple to yellow as the radical is scavenged by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. This activity is given as % DPPH radical-scavenging that is calculated in the equation:

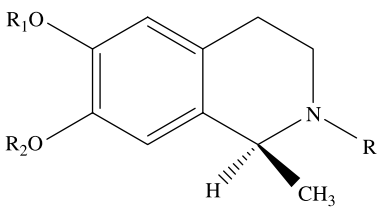
$$\%\ \text{DPPH radical scavenging} = \text{sample absorbance}/\text{control absorbance} \times 100.$$

The DPPH solution without sample was used as control. Acid ascorbic was used as positive control. The dose-response curve was obtained by plotting the percentage of inhibition versus the concentrations (Figure 2). Data presented in this study demonstrated that almost all the reported species possess free radical scavenging activity. As shown in Table III the alkaloid extract obtained from *S. oppositifolia* showed an interesting activity with an IC_{50} value of 16.3 μg/mL, while *S. soda* and *S. tragus* extracts exhibited an IC_{50} value of 24.3 μg/mL and 26.2 μg/mL, respectively. A dose-response relationship was observed for all *Salsola* extracts.

Table II. Tetrahydroisoquinoline alkaloids content of *Salsola* species.

	Percentage of alkaloids (% ± S.D.^)		
Alkaloid	*S. oppositifolia*	*S. soda*	*S. tragus*
Salsoline	5.8 ± 0.13	23.1 ± 0.09	36.5 ± 0.19
Salsolidine	10.5 ± 0.17	14.8 ± 0.04	17.7 ± 0. 12
N-Methylisosalsoline	6.3 ± 0.12	3.2 ± 0.03	1.1 ± 0.10
Carnegine	0.7 ± 0.12	tr	–

Percentage of alkaloids calculated with respect to alkaloid extract; ^S.D., Standard deviation ($n = 3$); tr, trace; –, not detected.



	R	R_1	R_2
Salsoline	H	H	CH_3
N-Methylisosalsolidine	CH_3	CH_3	H
Salsolidine	H	CH_3	CH_3
Carnegine	CH_3	CH_3	CH_3

Figure 1. Structures of identified alkaloids.

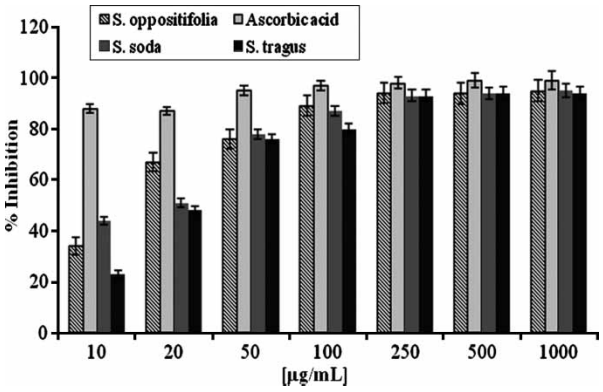


Figure 2. Dose-dependent antiradical activity of *Salsola* species alkaloid extracts. Each data represent the mean ± S.D. ($n = 3$).

Acetylcholinestearse and butyrylcholinesterase inhibition assay

Salsola alkaloid extracts are able to inhibit AChE and BChE enzymes in a dose-dependent manner (Figure 3). As shown in Table IV, *S. tragus* exerted the highest activity against AChE (IC_{50} of 30.2 µg/mL) and BChE (IC_{50} of 26.5 µg/mL). Moreover it is of interest to note that *S. soda* and *S. oppositifolia* extracts exhibited a selective inhibiting activity against BChE with IC_{50} values of 34.3 µg/mL and 32.7 µg/mL, respectively. *S. soda* and *S. oppositifolia* extracts were characterized by a major percentage of *N*-methylisosalsoline (3.2% and 6.3%, respectively) and carnegine (0.7% and trace, respectively). The alkaloid carnegine was not detected in *S. tragus* extract and *N*-methylisosalsoline is present with a percentage of 1.1%. The high level of alkaloids salsoline (36.5%) and salsolidine (17.7%) in ethyl acetate extract of *S. tragus* may be justify the major inhibiting activity of this *Salsola* species against both AChE and BChE enzymes. In fact, several studies have credited to these compounds with a very high cholinesterase activity [29–31]. Different isoquinoline alkaloids such as galanthamine and their derivatives, berberine, palmatine have been demonstrated to enhance cognitive functions in Alzheimer's patients [31]. Moreover, twenty-five iodomethylates of acetic, propionic, butyric, isobutyric and valeric esters of salsoline and salsolidine were studied as substrates and inhibitors of AChE from human erythrocytes and BChE from horse serum [35]. Cholinesterase inhibitors are the only approved drugs for treating patients with mild to moderately severe Alzheimer's disease on the basis of the cholinergic hypothesis. Cholinesterase inhibitors can restore the level of acetylcholine by inhibiting both AChE and BChE. enzymes.

Table III. Antiradical activity of *Salsola* species alkaloid extracts.

Sample	IC_{50} (µg/mL)
S. oppositifolia	16.3 ± 0.17
S. soda	24.3 ± 0.11
S. tragus	26.2 ± 0.09

Data are given as means ± S.D. ($n = 3$). Ascorbic acid (IC_{50} 2 µg/mL) was used as positive control.

Conclusions

The large number of natural compounds that are currently undergoing evaluation in clinical trials is a positive indicator that natural product discovery provides good value for human medicine with particular attention for chronic diseases. Alzheimer's disease is a devastating chronic disease that significantly increases healthcare costs and affects the quality of life of the afflicted patients and their caregivers. Most recently, advances in the discovery and development of plant-derived cholinesterase inhibitors have been assisted by new scientific technologies, including new production technologies, new design technologies and new developmental technologies. Moreover, new automated biological screening methods are also increasing the speed and efficiency of plant-derived new drugs discovery and development.

The present work reported for the first time the antioxidant and the acetyl- and butyryl-cholinesterase inhibitory activities of alkaloid extracts of three *Salsola* species. A dose-response relationship was observed for all samples. The different quantitative composition of alkaloid extracts modified the biological activities. Our results showed that *S. tragus* alkaloid extract has potent AChE and BChE inhibitory activity. On the contrary, *S. oppositifolia* and *S. soda* are characterized by a selective action against BChE, the other enzyme target considered a new approach for the AD treatment. Further *in vivo* investigations are necessary

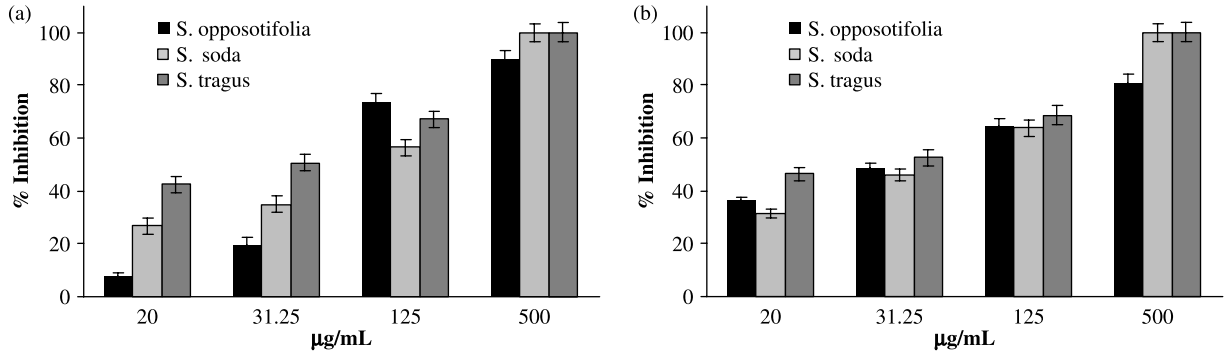


Figure 3. Dose-dependent inhibition of (a) acetylcholinesterase and (b) butyrylcholinesterase by *Salsola* species alkaloid extracts. Each data represent the mean ± S.D. ($n = 3$).

Table IV. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of *Salsola* species alkaloid extracts.

	IC_{50} (µg/mL)		
Sample	AChE	BchE	SI (BChE/AChE)
S. oppositifolia	70.0 ± 2.6⋆	32.7 ± 1.1⋆	0.5
S. soda	64.1 ± 1.2⋆	34.3 ± 1.4⋆	0.5
S. tragus	30.2 ± 1.4⋆	26.5 ± 1.0⋆	0.9
Physostigmine	0.2 ± 0.004	2.4 ± 0.02	14

IC_{50} values are mean ± S.D. ($n = 3$); One-way ANOVA Analysis: ⋆⋆⋆$p < 0.0001$; Dunnet's test: ⋆$p < 0.01$ (alkaloid extracts *vs* physostigmine).

in order to determine which alkaloid/s is/are responsible for the above-demonstrated effect.

Acknowledgements

The authors thank Dr. N.G. Passalacqua (Botany Department of University of Calabria, Italy) and Dr. L. Peruzzi (Biology Department, University of Pisa, Italy) for supplying the herb samples used in this study.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Pratico D, Delanty N. Am J Med 2000;109:577–585.
[2] Aruoma OI. Mutat Res 2003;523–524:9–20.
[3] Radi R, Peluffo G, Alvarez MN, Naviliat M, Cayota A. Free Rad Biol Med 2001;30:463–488.
[4] Mikiciuk-Olasik E, Szymański P, Zurek E. Indian J Exp Biol 2007;45:315–325.
[5] Hasselmo ME, Giocomo LM. J Mol Neurosci 2006;30:133–135.
[6] McGeer PL. The 12th J.A.F. Can J Physiol Pharmacol 1984;62:741–754.
[7] Darvesh S, Hopkins DA, Geula C. Neurosci 2003;4:131–138.
[8] Perry EK, Perry RH, Blessed G, Tomlinson BE. Appl Neurobiol 1978;4:273–277.
[9] Greig NH, Sambamurti K, Yu QS, Perry TA, Holloway HW, Haberman F, Brossi A, Ingram DK, Lahiri DK. Butyrylcholinesterase: Its selective inhibition and relevance to Alzheimer's disease. In: Giacobini E, editor. Butyrylcholinesterase: Its function and inhibition. London: Martin Dunitz Ltd; 2003.
[10] Tasker A, Perry EK, Ballard CG. Expert Rev Neurotherapeutics 2005;5:101–106.
[11] Ballard CG, Greig NH, Guillozet-Bongaarts AL, Enz A, Darvesh S. Curr Alzheimer Res 2005;2:307–318.
[12] Greig NH, Lahiri DK, Sambamurti K. Int Psychogeriatr 2002;14:77–91.
[13] Mukherjee PK, Kumar V, Mal M, Houghton PJ. Phytomedicine 2007;14:289–300.
[14] Rosenthal J. Steroids 1993;58:566–572.
[15] Al-Saleh FS, Ali H, Mirza M. Fitoterapia LXIV 1993;3:251–256.
[16] Nikiforov SB, Semenov AA, Syrchina AI. Pharm Chem J 2002;36:544–545.
[17] Loizzo MR, Tundis R, Statti GA, Passalacqua N, Peruzzi L, Menichini F. Nat Prod Res 2007;21:346–351.
[18] Tundis R, Loizzo MR, Statti GA, Menichini F. Pharmazie 2007;62:473–475.
[19] Tomàs F, Morenilla A, Barberàn FAT. Fitoterapia 1985;LVI:365–366.
[20] Zhao YX, Yao XB. Xue Xue Bao 2004;39:598–600.
[21] Swart P, Van der Merwe KJ, Swart AC, Todres PC, Hofmeyr JH. Planta Med 1993;59:139–143.
[22] Rizq AM. 1986., The Phytochemistry of the Flora of Qatar. Kingprint, Richmond, UK
[23] Shamma M. The isoquinoline alkaloids, chemistry and pharmacology. Verlag Chemie: Academic Press; 1972.
[24] Borkowski B, Drost K, Pasichowa B. Acta Polon Pharm 1959;16:57–61.
[25] Kuznetsova NN, Abdullaeva LK, Sadikov AA. Chem Nat Comp 2005;41:234–235.
[26] Vetulani J, Nalepa I, Antkiewicz-Michaluk L, Sansone M. J Neurol Transm 2001;108:513–526.
[27] Iwasa K, Moriyasu M, Tachibana Y, Kim HS, Wataya Y, Wiegrebe W, Bastow KF, Cosentino LM, Kozuka M, Lee KH. Bioorg Med Chem 2001;9:2871–2884.
[28] Aubry S, Pellet-Rostaing S, Fournier Dit Chabert J, Ducki S, Lemaire M. Bioorg Med Chem Lett 2007;17:2598–2602.

[29] Markmee S, Ruchirawat S, Prachyawarakorn V, Ingkaninan K, Khorana N. Bioorg Med Chem Lett 2006;16: 2170–2172.
[30] Kim DK, Lee KT, Baek NI, Kim SH, Park HW, Lim JP, Shin TY, Eom DO, Yang JH, Eun JS. Arch Pharm Res 2004;27: 1127–1131.
[31] Loizzo MR, Tundis R, Menichini F, Menichini F. Curr Med Chem 2008;15:1209–1228.
[32] Wang M, Li J, Rangarajan M, Shao Y, La Voie EJ, Ho Huang CT. J Agric Food Chem 1998;46:4869–4873.
[33] Ellman GL, Courtney KD, Andres V, Jr., Featherstone RM. Biochem Pharmacol 1961;7:88–95.
[34] Menachery MD, Lavanier GL, Wetherly ML. J Nat Prod 1986;49:745–778.
[35] Maizel' EB, Rozengart EV, Khakimov IuP, Abduvakhabov AA, Aslanov KhA. Biokhimiia 1978;43:1150–1156.