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Mouna Kchaou
Laboratory of Chemistry of Natural Substances, University of Sfax, Faculty of Sciences of Sfax, 3000, P.B"1171", 3000, Sfax, Tunisia.

Hichem Ben Salah
Laboratory of Chemistry of Natural Substances, University of Sfax, Faculty of Sciences of Sfax, 3000, P.B"1171", 3000, Sfax, Tunisia.

Raed Abdennabi
Laboratory of Plant Biotechnology Applied to Crop Improvement, Faculty of Science, Sfax University, BP 1171, 3000 Sfax, Tunisia

Ameni Walha
Laboratory of Chemistry of Natural Substances, University of Sfax, Faculty of Sciences of Sfax, 3000, P.B"1171", 3000, Sfax, Tunisia.

Noureddine Allouche
Laboratory of Chemistry of Natural Substances, University of Sfax, Faculty of Sciences of Sfax, 3000, P.B"1171", 3000, Sfax, Tunisia.

Correspondence:
Noureddine Allouche
Laboratory of Chemistry of Natural Substances, University of Sfax, Faculty of Sciences of Sfax, 3000, P.B"1171", 3000, Sfax, Tunisia.

Antioxidant, antibacterial and antiacetylcholinesterase activities of *Phalaris canariensis* from Tunisia

Mouna Kchaou, Hichem Ben Salah, Raed Abdennabi, Ameni Walha, Noureddine Allouche

Abstract
This study evaluates the antioxidant, antibacterial and antiacetylcholinesterase activities as well as phenolic contents of various extracts from *Phalaris canariensis*. The results showed that the methanol extract is endowed with the highest antiacetylcholinesterase activity (PI = 65.0%) and the best radical scavenging property with IC_{50}=0.12 mg/mL. Meanwhile, this extract was found to have the highest phenolic content (190.58 mg gallic acid equivalents/g extract). Moreover, the ethyl acetate extract exhibited the best reducing power effect and the highest total antioxidant capacity (330.9 mg vitamin E/mg extract). In addition, the best antibacterial effect was observed for the methanolic extract against *Micrococcus luteus*. It was noticed that the extents of antiacetylcholinesterase, antibacterial and antioxidant activities of *Phalaris canariensis* extracts are in accordance with their phenolic compounds.

Keywords: *Phalaris canariensis,* antioxidant, antibacterial, antiacetylcholinesterase, polyphenols, flavonoids.

Introduction
Medicinal plants are good sources of active natural products that differ widely in terms of structure and biological properties, so they can be used for various applications, especially as food additives and health promoting ingredients. For the reason, during last few decades, they have been become a subject for study of bioactive compounds [1]. Many of these plants and their products are also excellent sources for phytochemicals and have been found to possess a variety of biological activities including antioxidant, antibacterial and antiacetylcholinesterase activities [2]. However, the oxidative stress which involves the production of reactive oxygen species (ROS), is the basis for chronic diseases and aging is being reexamined. Accumulating evidence suggests that ROS exert essential metabolic functions and that removal of too many ROS can upset cell signaling pathways and actually increase the risk of chronic disease [3].
In recent decades, there has been growing interest in finding natural antioxidants in plants because they inhibit oxidative damage and may consequently prevent aging and neurodegenerative diseases [4]. Antioxidants are widely used in the food industry as potential inhibitors of lipid peroxidation [5]. However, many synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may be accumulated in the body resulting in liver damage and carcinogenesis [6]. For this reason, more attention has been paid to natural non-toxic antioxidants in an effort to protect the human body from free radicals and retard the progress of many chronic diseases.
Neurodegenerative diseases are nowadays also one of the major concerns due to the increase in life expectations. Among these include Parkinson's disease (PD), Huntington's disease (HD) and Alzheimer's disease (AD). Alzheimer's disease (AD) is a devastating neurological disorder characterized by a profound degradation of cognitive function and a progressive loss of memory [7]. Medical researchers have generally confirmed that the loss of acetylcholine in brain ultimately leads to the appearance of AD [8]. One of the most promising approaches for treating this latter disease is to improve the acetylcholine level in the brain using acetylcholinesterase (AChE) inhibitors [9]. Inhibition of acetylcholinesterase (AChE), the key enzyme in the breakdown of Acetylcholine (ACh), is considered to be a promising for the treatment of Alzheimer's disease (AD). However, only tacrine, donepezil and rivastigmine were used for treatment of AD and have been approved by the food and drug administration in the United States [10], but this synthetically drugs have been reported to cause problems associated with bioavailability and gastrointestinal disturbances [11]. Therefore, the search for new AChE inhibitors, particularly from natural products, with higher efficacy continues.

In terms of biodiversity, the flora of Tunisia represents one of the richest areas in the mediterranean and comprises a very important resource of medicinal plants.

The canary grass seed (*Phalaris canariensis* L.) is a shrubby plant belonging to the Poaceae family with production practices and life cycle similar to those of other winter cereal crops such as wheat and oats [12, 13]. This is a perennial rhizomatous psychotropic effect. It is spread throughout the entire northern hemisphere, particularly in Asia, Europe, North America and North Africa. *Phalaris canariensis* commonly grows on soils rich in nutrients with a slightly acidic to neutral pH, and also on the wetlands wet. Traditionally, *Phalaris canariensis* is also used in folk medicine for diabetes and hypertension treatments. However there is no scientific information about the possible bioactive compounds responsible for such effects.

The present study was undertaken to examine antioxidant property of *Phalaris canariensis* extracts by investigating the DPPH radical scavenging, reducing power and total antioxidant capacity effects as well to evaluate their antiacetylcholinesterase and antibacterial activities. In addition, the total phenolic and flavonoid contents of the extracts were determined.

Materials and Methods

Collection of plant Material

Mature seeds of *Phalaris canariensis* were collected from region in Tunisia: Beja (a city of the northwest of Tunisia with a semi-arid climate) in December 2013. A voucher specimen (Number LCSN 132) has been deposited in the Herbarium of the Laboratory of Chemistry of Natural Substances, Faculty of Sciences, Sfax University, Tunisia.

Preparation of the extracts

The powdered seeds (300 g) of *Phalaris canariensis* were extracted separately by ultrasonication bath at 40 °C, using three different solvents: Hexane, Ethyl acetate and Methanol (500 ml each).

After filtration and evaporation, three fractions were obtained: hexane (19.32g), ethyl acetate (5.40g) and methanol (23.61g). These fractions were stored in darkness at 4 °C.

Determination of Phenolics Content

The total phenolics content in extracts was determined with Folin-Ciocalteau reagent using the method of Chen *et al*. [14]. A standard curve must be first plotted using gallic acid as a standard. Different concentrations of gallic acid were prepared in methanol, and their absorbances were recorded at 750 nm. 100 µL of diluted sample was added to 2 mL of 2% Na_2CO_3 aqueous solution. After 2 min, 100 µL of 50% Folin-Ciocalteau reagent was added. The final mixture was shaken and then incubated, at room temperature in the dark, for 30 min. The absorbance of all samples was measured at 750 nm, and the results are expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract).

Determination of Flavonoids Content

The total flavonoids content in extracts was determined according to the procedure of Djeridane *et al*. [15], using a method based on the formation of complex flavonoid aluminium, having the maximum absorbance at 430 nm. Quercetin was used to make the calibration curve. About 1 mL of diluted sample was mixed with 1 mL of 2% aluminium trichloride ($AlCl_3$) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm and the total flavonoids content was expressed in mg quercetin equivalents per gram of extract (mg QE/g extract).

DPPH radical scavenging assay

The hydrogen atom or electron donation ability of the corresponding extracts was measured from the bleaching of the purple-coloured methanol solution of DPPH. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging effect was evaluated following the procedure described in a previous study (Tepe B. *et al*.) [16]. Fifty microliters of various concentrations of extract in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical, DPPH, in percent (PI %) was calculated in the following way:

$$PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Extract concentration providing 50% inhibition of DPPH (IC_{50}) was calculated from the graph plotted of inhibition percentage against extract concentration. The synthetic antioxidant reagents butylate hydroxytoluene (BHT) and vitamin E are used as positive control and all tests were carried out in triplicate.

Reducing power

The method of A. Yildirim *et al*. [17] was used to assess the reducing power of the extracts. 1ml of different concentrations of extracts (5, 10, 25, 50, 100 µg/ml) was mixed with 2.5 ml of a 0.2 M sodium phosphate buffer (pH = 6.6) and 2.5 ml of 1% potassium ferricyanide ($K_3Fe(CN)_6$), and incubated in a water bath at 50°C for 20 min. Then, 2.5 ml of 10% trichloroacetic acid were added to the mixture that was centrifuged at 650 g for 10 min. The supernatant (2.5 ml) was then mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride solution. The absorbance of the mixture was measured at 700 nm. Increased absorbance of the mixture indicated increased reducing power. A standard curve was prepared using various concentration of vitamin E.

Total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto, Pineda and Aguilar [18]. The assay is based on the reduction of Mo(VI)–Mo(V) by the extracts and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml of sample at different concentrations was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95 °C for 90 min. Then the absorbance of the solution was measured

at 695 nm using a spectrophotometer UV–Vis against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of vitamin E.

Determination of Antibacterial Activity

The bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37 ˚C for 18 to 24 h prior to seeding onto the nutrient agar. One or several colonies of the respective bacteria were transferred into API suspension medium (bio Merieux) and adjusted to 0.5 McFarland turbidity standards with a Densimat (bio Merieux) (Saïdana *et al*., 2008; Hichri *et al*., 2003) [19, 20]. The inocula of the respective bacteria were streaked into MH agar plates using a sterile swab and were then dried at 37 ˚C during 15 min. A sterile filter disc having 6 mm of diameter was placed at the surface of MH agar and 5 µl of the extract was dropped onto each Whatman paper disc (Bel Haj Khether *et al*., 2008) [21]. The treated Petri dishes were incubated at 37 ˚C for 18 to 24 h. The antibacterial activity was evaluated by measuring the clear zone surrounding the Whatman paper. Standard discs of the antibiotic ampicillin (BIO-RAD) were applied as a positive antibacterial controls.

Acetylcholinesterase enzyme inhibitory activity

Inhibition of acetylcholinesterase biosynthesis by plant extracts was investigated using micro-plate assays. The enzyme activity is measured by observing the increase of a yellow colour produced from thiocholine when it reacts with the dithiobis nitrobenzoic ion (DTNB).

The assay for measuring AChE activity is based on the assay described by Ellman *et al*. [22] and Ingkaninan *et al*. [23] with modifications. Briefly, 125 µl of 3 mM DTNB, 50 µl of sodium phosphate buffer (pH 8.0), 25 µl of sample dissolved in DMSO and 25 µl of 0.5 U/ml AChE were added in a 96-well microplate and incubated for 15 min at 25 ˚C. The reaction was then initiated with the addition of 25 µl of acetylthiocholine iodide (ATCI). The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 405 nm.

The microplate was then read at 405 nm every 3 min by a CERES UV 900 C microplate reader (Bio-Tek Instrument, USA). The velocities of the reactions were measured. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer without any inhibitor. Inhibitory activity was calculated from 100 subtracted by the percentage of enzyme activity. Every experiment was done in triplicate

The percent of inhibition was calculated as following:

$$PI\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control and A_{sample} is the absorbance of the test sample. The $IC_{50}$was calculated by log-probit analysis. Tacrine was used as positive control.

Statistical analysis

The data was analysed using the statistical software program SPSS 18.0 for Windows. All results were given as mean ± standard division (SD). The potential correlation among the acetylcholinesterase inhibitory, antioxidant capacity, total phenol and flavonoid contents were analysed and differences at $P < 0.05$ were considered as "significant", $P < 0.01$ " highly significant" and $P > 0.05$ " non-significant".

Results and Discussion

Extraction yield

The percentage yields of plant extracts are shown in Figure 1. The extraction yield obtained with methanol (7.87%) is higher than those obtained with hexane (6.44%) and ethyl acetate (1.8%). This is due to the affinity between polarity of solvents and extracted compounds [24, 25].

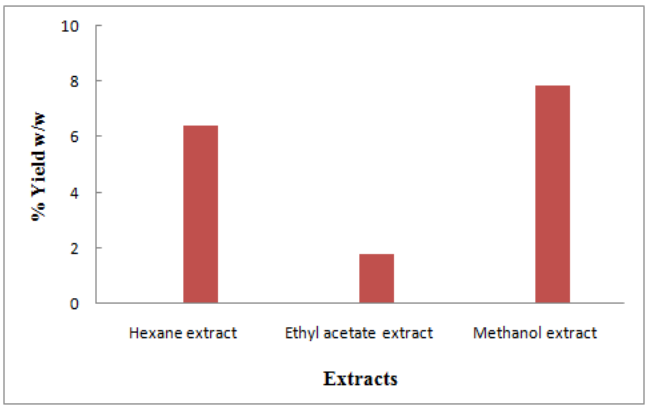

Fig 1: Percentage yields of different extracts from *Phalris canariensis*

Total Phenols Content (TPC) and total flavonoids content (TFC)

Phenolic compounds (phenols and flavonoids) received considerable attention because of their potential antioxidant activity [26, 29]. These compounds are known for their ability of scavenging free radicals and active oxygen species such as single oxygen [30]. Based on the absorbance values of the extracts solutions compared with the standard solutions of gallic acid and quercetin, the total phenolic and flavonoid contents are shown in Table1. Except the hexane extract, which contain low amounts of phenolic compounds, the other extracts were found to be rich in these compounds. As shown in Table1, methanol extract showed the highest amount of phenolic compounds (190.58 mg GAE/g extract) followed by ethyl acetate (181.06mg GAE/g extract) and hexane (36.40 mg GAE/g extract) extracts.

Table1: Total phenolic and flavonoid contents in different extracts of *Phalaris canariensis*

Extracts	Total phenolics (mg GAE/g extract)	Total Flavonoids (mg Q/g extract)
Hexane	36.40 ± 0.72	3.24 ± 0.01
Ethyl Acetate	181.06 ± 3.62	21.76 ± 0.10
Methanol	190.58 ± 3.81	9,72 ± 0.04

Total flavonoids content varied from 3.24 ± 0.01 to 21.76 ± 0.1 mg/g extract. The order of flavonoid contents in the extracts is: Ethyl acetate > Methanol > Hexane. These results showed that *Phalaris canariensis* extracts may be

suggested as a potential source of natural phenolic compounds which are endowed with several activities especially antioxidant one [31].

DPPH radical-scavenging assay

The free radical scavenging activity of *Phalaris canariensis* extracts was evaluated using the DPPH test. This test aims to measure the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picryl hydrazil (DPPH) formed in solution by donation of a hydrogen atom or an electron [16]. DPPH gives a strong absorption band at 517 nm in visible spectroscopy. The stable free radical DPPH has been widely used to test the free radical-scavenging capacity of various antioxidants [27]. If the extracts have the capacity to scavenge the DPPH free radical, the initial purple solution will change to a yellow color due to the formation of diphenyl picryl hydrazine. In the DPPH assay, the radical scavenging ability of the extracts and the positive controls (BHT and vitamin E) was analyzed in triplicates. DPPH scavenging activities of *Phalaris canariensis* extracts and standard antioxidants are given in (Table 2). IC_{50} values of extracts stood between 0.12 and 0.22 mg/mL. The highest antioxidant activity (IC_{50} = 0.12 mg/mL) was related to the methanol extract. The order of DPPH scavenging activity of extracts was: Methanol > Ethyl acetate > Hexane. Although the IC_{50} of various extracts were significantly lower than those of BHT and vitamin E, it was evident that the extracts show the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. This radical scavenging activity of extracts could be related to their phenolic compounds, thus contributing to their electron transfer/hydrogen donating ability [32].

Table 2: Antioxidant activity of extracts from *Phalaris canariensis*

Extracts	IC_{50} (mg/mL)
Hexane Extract (HE)	0.22±0.024
Ethyl Acetate Extract (EAE)	0.15±0.03
Methanol Extract (ME)	0.12±0.04
BHT	0.017±0.340
Vitamin E	0.026±0.520

Reducing power

The reducing power of an extract is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to green and blue depending on the reducing power activity of test specimen. Therefore, in this study, reducing activity was determined based on the ability of extracts to reduce a Fe^{3+} ferricyanide complex to form a Fe^{2+} ferrous complex. The amount of Fe^{2+} was monitored by measuring the formation of perl's prussian blue at 700 nm [33]. The reducing power of various extracts of *Phalaris canariensis* is given in Figure 2. The reducing power of the different extracts was found to decrease in this order: Ethyl acetate > Methanol > Hexane. This result suggests that extracts have a good ability to donate electrons to reactive free radicals, converting them into more stable products.

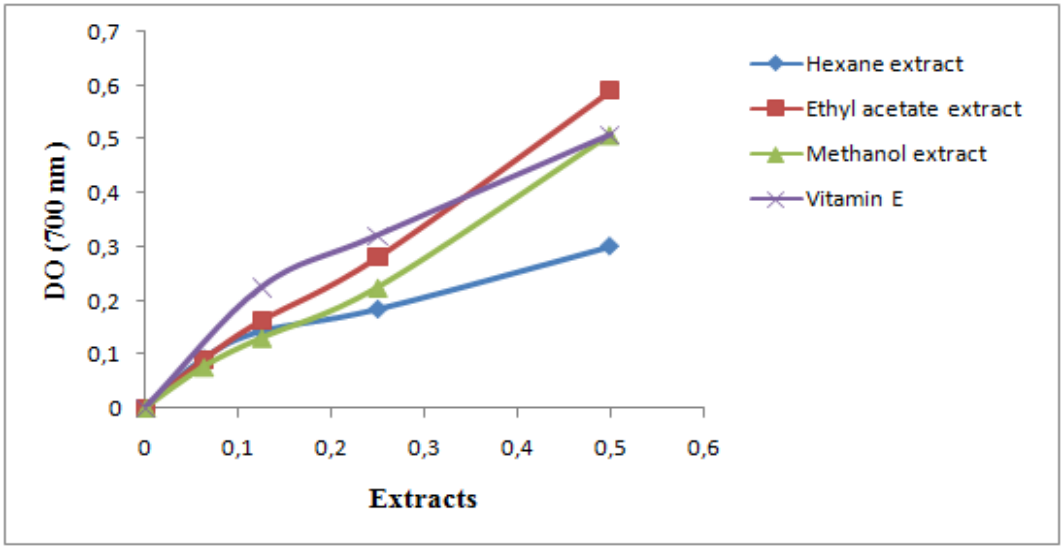

Fig 2: reducing power of *Phalaris canariensis* extracts

This result can be related to phenolic compounds which were quantified in the extracts. Those compounds have a good ability to donate electrons to reactive free radicals, converting them into more stable products and terminating the free radical chain reaction [34].

Total antioxidant capacity

The total antioxidant capacity of various extracts of *Phalaris canariensis* is given in Figure 3 and it is expressed as the number of equivalents of vitamin E. The phosphomolybdenum method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm [35, 36]. High absorbance values indicated that the sample possesses significant antioxidant activity. The total antioxidant activity was analyzed by phosphomolybdenum method based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of an electron transfer occurring in this assay depends upon the structure of the antioxidant [37].

The antioxidant capacity value of ethyl acetate extract (330.9 mg of antioxidant /g of extract) was higher than that of

methanol (326.1 mg of antioxidant /g of extract) and hexane (59.8 mg of antioxidant /g of extract) extracts. This result is in agreement with the phenolic contents variation in the extracts (Table 1).

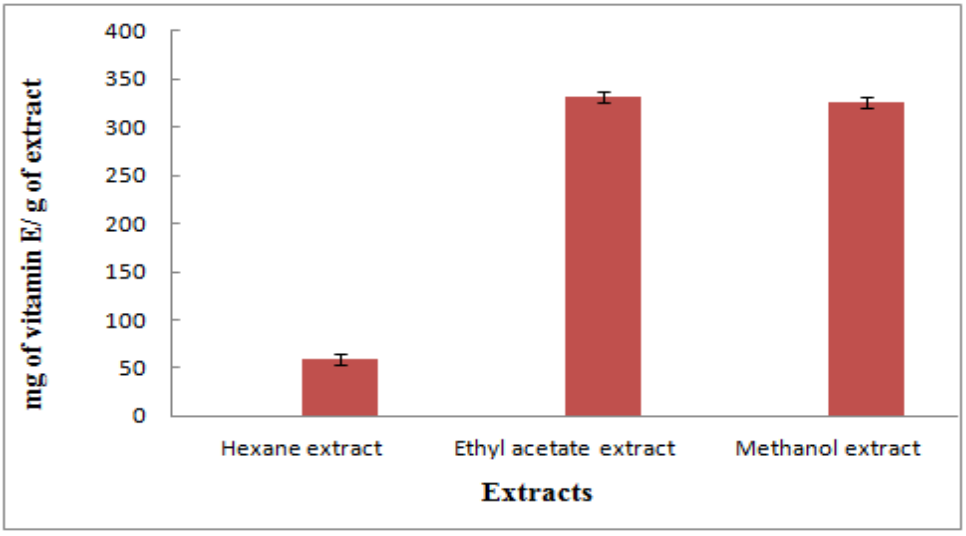

Fig 3: Total antioxidant capacity of *Phalaris canariensis* extracts

Thus, *Phalaris canariensis* seed extracts could be used as natural antioxidant agents. This property may contribute to the reduction of the risk of chronic diseases such as cancer and cardiovascular disease and could be useful for the food drug industry [38].

Acetylcholinesterase activity

Many plants have been used traditionally to enhance cognitive function and to alleviate other symptoms associated nowadays with Alzheimer's disease (AD) [39]. Acetylcholinesterase (AChE) inhibition is an important drug treatment strategy against AD and, recently, there has been considerable interest in finding naturally acetylcholinesterase inhibitors to replace synthetic drugs such tacrine and donepezil (having some adverse effects) [40]. For this purpose, our data revealed that *Phalaris canariensis* could serve as inhibitor against cholinesterase enzyme family. The acetylcholinesterase (AChE) enzyme inhibition of the extracts was determined using Ellman's colorimetric method in 96-welled microplate.

The results obtained through this study of acetylcholinesterase enzyme inhibitory activity of the tested extracts were shown in Figure 4. AChE inhibitory activity of extracts was found to increase dose-dependently, the results were expressed as percentage inhibition values. At the concentration of 1 mg ml^{-1}, the methanol extract give a strong percentage of inhibition (65.0%), however the ethyl acetate and hexane extracts showed moderate percentages of inhibition (25.4 and 45.8 %, respectively). Tacrine, used as a standard, shows inhibition of acetylcholinesterase with inhibition percentage of 80.5% at a concentration of 0.1 mg ml^{-1}. The inhibitory action of *Phalaris canariensis* extracts against acetylcholinesterase activity would be related to their content of polyphenols and flavonoids. Recently, it was reported that these compounds have been shown to possess antiacetylcholinesterase properties [41, 45]. In our study, the *in vitro* inhibition of acetylcholinesterase by *Phalaris canariensis* extracts is performed for the first time. The obtained results indicate that these extracts may offer great potential for the treatment of Alzheimer Disease.

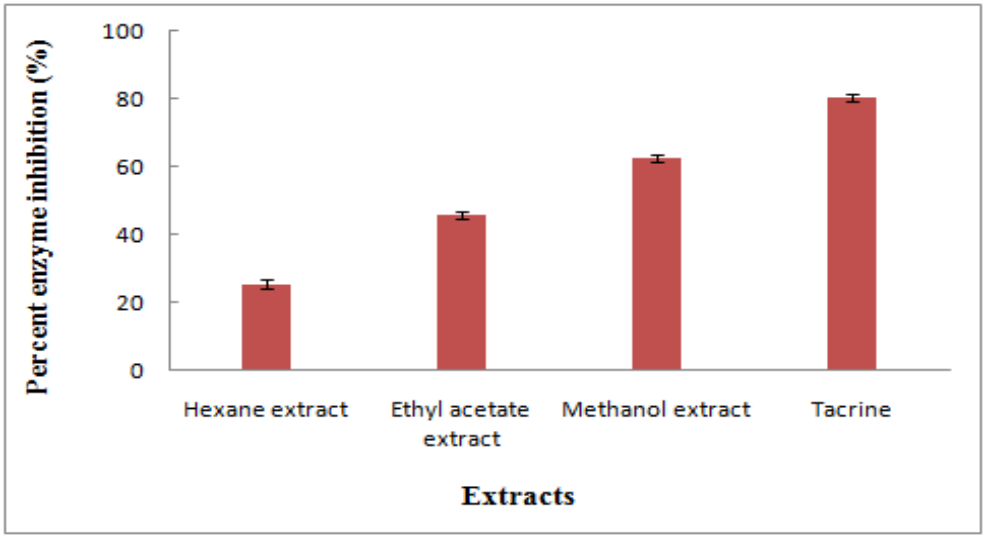

Fig 4: The inhibitory activity of acetylcholinesterase of *Phalaris canariensis* extracts

Antibacterial Activity

Antibacterial activity of *Phalaris canariensis* extracts was evaluated against a set of significant bacterial strains, including both Gram-positive and Gram-negative. The inhibitory effect on bacterial growth was determined using agar disc diffusion assay [46, 47]. The obtained results from this test are summarized in Table 3. The extracts showed an interesting antibacterial activity especially against gram-positive bacteria. The results indicated that methanol and ethyl acetate extracts of *Phalaris canariensis* showed a high antibacterial activity against *Micrococcus luteus*. In addition, *Bacillus subtilis*, a highly pathogenic strain, has a high sensitivity to the hexane and ethyl acetate extracts. This high activity may be related to the presence of phenolic compounds. These compounds are known for their antibacterial activity [48]. On the other hand, the extracts were not active against *Salmonella sp, Escherichia coli 25922* and *Enterococcus faecalis*. The highest resistance in Gram (-) bacteria might be explained by the presence of their outer membrane covering the cell wall, which limits the diffusion of hydrophobic compounds through its lipopolysaccharide coating. Most studies have reported that plant extracts generally are more active against the Gram-positive bacteria than the Gram-negative bacteria [49, 50].

Table 3: Antibacterial activity of *Phalaris canariensis* extracts using agar disc.

Strains	DD [a]	DD [b]	DD[c]	DD[d]
Bacterial strains Gram (+)				
Listeria monocytogenes	na	na	11	19
Micrococcus luteus	na	15	27	22
Bacillus subtilis	20	24	na	26.0
Bacterial strains Gram (-)				
Salmonella sp	15	na	na	16
Escherichia coli 25922	na	na	na	22
Enterococcus faecalis	na	na	na	14

Average ± Standard Deviation were obtained from three different experiments.

[a] DD: Disc Diameter of inhibition (halo size) in (mm), hexane extract 100 µg/disc.

[b] DD: Disc Diameter of inhibition (halo size) in (mm), ethyl acetate extract 100 µg/disc.

[c] DD: Disc Diameter of inhibition (halo size) in (mm), methanol extract 100 µg/disc.

[d] DD: Disc Diameter of inhibition zone of ampicillin (10 µg/disc), was used as positive control for bacteria.

n.a.: not active.

Conclusion

In conclusion, our investigation clearly demonstrated for the first time that crude extracts from *Phalaris canariensis* exhibited a powerful antioxidant activity. Moreover, these extracts are endowed with a high antibacterial activity especially against gram-positive bacteria. Furthermore, the antiacetylcholinesterase assay showed that methanol extract of *Phalaris canariensis* is endowed with a potent activity. On the other hand, this investigation showed a notable relationship between phenolic contents in the extracts and their antibacterial, antiacétylcholinesterase and antioxidant activities. Our study provides the possible pharmacologic rationale to the medicinal use of *Phalaris canariensis* in the development of antibacterial and antialzheimer drugs.

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Conflict Of Interest Statement

We declare that we have no conflict of interest.

References

1. Loziene K, Venskutonis PR, Sipailiene A, Labokas J. Radical scavenging and antibacterial properties of the extracts from different Thymus pulegioides L. chemotypes. Food Chem 2007; 103:546e549.
2. Bekir J, Mars M, Souchard JP, Bouajila J. Assessment of antioxidant, anti-inflammatory, anti-cholinesterase and cytotoxic activities of pomegranate (*Punica granatum*) leaves. Food Chem Toxicol 2013; 55:470-475.
3. Su B, Wang X, Nunomura A, Moreira PI, Lee HG, Perry G *et al.* Oxidative stress signaling in Alzheimer's disease. Curr Alzheimer Res 2008; 5:525-532.
4. Fusco D, Colloca G, Lo Monaco MR, Cesari M. Effects of antioxidant supplementation on the ageing process. Clin Interventions in Aging 2007; 2:377-387.
5. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1- picrylhydrazyl method. Food Chem 2009; 112:654-658.
6. Valentao P, Fernandes E, Carvalho F, Andrade PB, Scabra RM, Bastos ML. Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. J Agric Food Chem 2002; 50:4989-4993.
7. McGeer PL, McGeer EG. Alzheimer's disease: arthritis of the brain. Drug News & Perspectives 1995; 8:80-83.
8. Zou L, Wang LJ, Cheng YQ, Li LT, Eizo T, Koji Y. Acetylcholinesterase inhibitory activities of Douchi, a traditional Chinese food. Food Science 2006; 27(3):87-90.
9. Saify ZS, Sultana N. Role of Acetylcholinesterase Inhibitors and Alzheimer Disease. Drug Design and Discovery in Alzheimer's Disease, 2014, 387-425.
10. Rossignol DA, Frye RE. The Use of Medications Approved for Alzheimer's Disease in Autism Spectrum Disorder: A Systematic Review. Front Pediatr 2014: 2:87
11. Mehta M, Adem A, Sabbagh M. New Acetylcholinesterase Inhibitors for Alzheimer's Disease. Intern J Alzheimer's Disease, 2012. doi:10.1155/2012/7289832.
12. Takagi T, IIDA T. Antioxidant for Fats and Oils from Canary Seed: Sterol and Triterpene Alcohol Esters of Caffeic Acid. JAOCS, 1980.
13. El-Sayed M. Abdel-Aal, Pierre Hucl, Carol Ann Patterson, Danielle Gray. Fractionation of Hairless Canary Seed (Phalaris canariensis) into Starch, Protein, and Oil. J Agric Food Chem 2010; 58:7046-7050.
14. Chen HY, Lin YC, Hsieh CL. Evaluation of antioxidant activity of aqueous extract of some selected nutraceutical herbs. Food Chem 2007; 104:1418-1424.
15. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some algerian

medicinal plants extracts containing phenolic compounds. Food Chem 2006; 97:654-660.
16. Tepe B, Sokmen M, Akpulat HA, Sokmen A. Screening of the antioxidant potentials of six Salvia species from Turkey. Food Chem 2006; 95:200-204
17. Yildirim A, Mavi A, Kara AA. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J Agric Food Chem. 2001; 49:4083-4089.
18. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem 1999; 269:337-341.
19. Saïdana D, Mahjoub S, Boussaada O, Chriaa J, Mahjoub MA, Chéraif I *et al*. Antibacterial and Antifungal Activities of the Essential Oils of two Saltcedar Species from Tunisia. J Am Oil Chem Soc. 2008; 85:817-826.
20. Hichri F, Ben Jannet H, Cheriaa J, Jegham S, Mighri Z. Antibacterial activities of a few prepared derivatives of oleanolic acid and of other natural triterpenic compounds. Comptes Rendus Chimie, 2003; 6:473-483.
21. Bel Haj Khether F, Ammar S, Saidana D, Daami-Remadi M, Cheriaa J, Liouane K *et al.* Chemical composition, antibacterial and antifungal activities of Trichoderma sp. growing in Tunisia. Annals of Microbiology 2008; 58:303-308.
22. Ellman GL, Lourtney DK, Andres V, Gmelin G. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7:88-95.
23. Ingkaninan K, De Best CM, Irth H, Van Der Heijden R, Hofte AJP, Karabatak B *et al.* High performance liquid chromatography with on-line coupled UV-mass spectrophotometric-biochemical detection for identification of acetyl cholinesterase inhibitors from natural products. J Chromatogr A. 2000; 872:61-73.
24. Dash S, Nath LK, Bhise S, Bhuyan S. Antioxidant and antimicrobial activities of Heracleum nepalense D Don root. Trop J Pharmaceut Res. 2005; 4(1):341-347.
25. Padalia H, Moteriya P, Satasiya R, Chanda S. *In vitro* free radical scavenging activity Nerium indicm, Peltophoram pterocarpum and Rosa spps. flower extracts. Asian J Pharmaceut Clin Res. 2015; 8(1):91-97.
26. Suhartonoa E, Vianib E, Rahmadhan MA, Gultom IS, Rakhman MF, Indrawardhanac D. Total flavonoid and Antioxidant Activity of Some Selected Medicinal Plants in South Kalimantan of Indonesian. APCBEE Procedia 2012; 4:235-239.
27. Pavithra K, Vadivukkarasi S. Evaluation of free radical scavenging activity of various extracts of leaves from Kedrostis foetidissima (Jacq.) Cogn. Food Sci Human Wellness 2015; 4:42-46.
28. Khlif I, Jellali K, Michel T, Halabalaki M, Skaltsounis AL, Allouche N. Characteristics, Phytochemical Analysis and Biological Activities of Extracts from Tunisian Chetoui *Olea europaea* Variety. Hind Pub Corp J Chem. 2015. doi:org/10.1155/2015/418731.
29. John JA, Shahidi F. Phenolic compounds and antioxidant activity of Brazil nut (Bertholletia excelsa). J func foods. 2010. doi: 10.1016/j.jff.2010.04.008.
30. Lahlou FA, Hmimid F, Loutfi M, Bourhim N. Antioxidant Activity Phenolics Flavonoids and Proanthocyanidins Content of Senecio anteuphorbium
31. Keskes H, Mnafgui K, Hamden K, Damak M, El Feki A, Allouche N. *In vitro* Anti-diabetic, Anti-obesity and Antioxidant proprieties of Juniperus phoenicea L. leaves from Tunisia. Asian Pacific J Tropical Medicine 2014; 4:649-655.
32. Sacan O, Yanardag R. Antioxidant and antiacetylcholinesterase activities of chard (Beta vulgaris L. var. cicla). Food Chem Toxicol 2010; 48:1275-1280.
33. Ebrahimzadeh MA, Nabavi SM, Nabavi SF, Bahramian F, Bekhradnia AR. Antioxidant and free radical scavenging activity of H. officinalis, L. Var. angustifolius, V. odorata, B. hyrcana and C. speciosum. Pak J Pharm Sci 2010; 23:29-34.
34. Sun L, Zhang J, Lu X, Zhang L, Zhang Y. Evaluation to the antioxidant activity of total flavonoids extract from persimmon (*Diospyros kaki* L.) leaves. Food Chem Toxicol 2011; 49:2689-2696.
35. Zhao HF, Fan W, Dong JJ, Lu J, Chen J, Shan LJ *et al.* Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chemistry 2008; 107:296-304.
36. Prasad KN, Yang B, Yang SY, Chen YL, Zhao MM, Ashraf M *et al.* Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi sinensis Sonn.)* seeds. Food Chem 2009; 116:1-7.
37. Kammoun M, Miladi S, Ben Ali Y, Damak M, Gargouri Y, Bezzine S. *In vitro* antioxidant study of the PLA2 inhibition and antioxidant activities of *Aloe vera* leaf skin extracts. Lipids Health Dis, 2011. doi: 10.1186/1476-511X-10-30.
38. Selappan S, Akoh CC, Krewer G. Phenolic compounds and antioxidant capacity of georgia-grown blueberries and blackberries. J Agric Food Chem. 2002; 50:2432-2438.
39. Natarajan S, Shunmugiah KP, Kasi PD. Plants traditionally used in age-related brain disorders (dementia): an ethanopharmacological survey. Pharma Biol 2013; 4:492-52.
40. Chatiipakorn S, Pongpanparadorn A, Pratchayasakul W, Pongchaidacha A, Ingkaninan K, Chatiipakorn N. *Tabernaemontana divaricata* extract inhibits neuronal acetylcholinesterase activity in rats. J Ethnopharmacol. 2007; 110:61-68.
41. Ji HF, Zhang HY. Multipotent natural agents to combat Alzheimer's disease: Functional spectrum and structural features. Acta Pharmacol 2008; 29:143-151.

42. Farlow MR. The SERAD study of the safety and efficacy of galantamine in severe Alzheimer's disease. The lancet neurology, 2009, 22-23.
43. Zhao Y, Dou J, Wu T, Aisa HA. Investigating the Antioxidant and Acetylcholinesterase Inhibition Activities of Gossypium herbaceam. Molecules 2013; 18:951-962.
44. Moyo M, Ndhlala AR, Finnie JF, Staden JV. Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of Sclerocarya birrea and Harpephyllum caffrum (Anacardiaceae) extracts. Food Chem 2010; 123:69-76.
45. Cao J, Xia X, Dai X, Xiao J, Wang Q, Marobela KA, Okatch H. Flavonoids profiles, antioxidant, acetylcholinesterase inhibition activities of extract from Dryoathyrium boryanum (Willd.) Ching. Food Chem Toxicol 2013; 55:121-128.
46. Pérez C, Agnese AM, Cabrera JL. The essential oil of Senecio graveolens (Compositae): chemical composition and antimicrobial activity tests. J Ethnopharmacol. 1999; 66:91-96.
47. Bagamboula CF, Uyttendaele M, Debevere J. Acid tolerance of Shigella sonnei and Shigella flexneri. J Appl Microbiol. 2002; 93:479-486.
48. Naïma Guendouze-Bouchefa, Khodir Madani, Mohamed Chibane, Lila Boulekbache-Makhlouf, Didier Hauchardc, Martin Kiendrebeogo, Caroline Stévignye, Philippe Ndjolo Okusaf, Pierre Duez. Phenolic compounds, antioxidant and antibacterial activities of three Ericaceae from Algeria Industrial. Crops and Products 2015; 70:459-466.
49. Gutiérrez-Larraínzar M, Rúa J, Caro Castro CD, Arriaga DD, García- Armesto MR, Valle PD. 2012. Evaluation of Antimicrobial and Antioxidant Activities of Natural Phenolic Compounds against Foodborne Pathogens and Spoilage Bacteria. Food Control, 26, 555-563.
50. Salmanian S, Sadeghi Mahoonak AR, Alami M, Ghorbani M. Phenolic Content, Antiradical, Antioxidant, and Antibacterial Properties of Hawthorn (Crataegus elbursensis) Seed and Pulp Extract. J Agr Sci Tech. 2014; 16:343-354.