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SCREENING OF COMMONLY USED JORDANIAN SPICES FOR INHIBITORY ACTIVITY AGAINST ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN ALZHEIMER'S DISEASE

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Abstract

The aim of this study was to evaluate the ability of commonly used Jordanian spices to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) for the management of Alzheimer's disease (AD). Both aqueous and methanolic extracts (1:1; v/v) of 30 spices were prepared and evaluated using a thin-layer chromatography (TLC) assay and Ellman's spectrophotometric method. TLC bioautography revealed 3 active aqueous extracts and 11 active methanolic extracts were able to inhibit AChE and BuChE. The methanolic extracts of 11 spices were able to inhibit either AChE or BuChE, or both (> 60% inhibition at a concentration of 100 µg/mL). The most active extracts against both enzymes were found in turmeric, cumin, dill, lemon grass, lemon verbena, sumac, fennel, white and black pepper, cinnamon and cardamom. Both methanolic and aqueous extracts of cumin, dill and fennel were active against both enzymes. The results indicate that the intake of theses spices and phytochemicals can greatly impact the onset and progression of chronic neurodegenerative diseases such as AD. Public health efforts should be directed at increasing the consumption of spices as part of a regular diet, which should also include consumption of protective phytonutrients. Of these the most common Jordanian herbs and spices, turmeric and cumin have the greatest potential for lowering the risk of Alzheimer's disease.

Keywords: *Acetylcholinesterase, Butyrylcholinesterase, Jordanian spices and herbs, Alzheimer's disease (AD.)*

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the progressive deterioration of memory and other cognitive functions [1]. Epidemiological data reveal 46.8 million people worldwide were living with dementia in 2015, which is predicted to reach 131.5 million by 2050 [2]. In addition to being a multifactorial and complex neurodegenerative brain disorder, the exact pathophysiology of AD is not entirely known. However, several pathogenic mechanisms of AD have been suggested: deficits in the cholinergic system, accumulation of beta-amyloid in the brain, oxidative stress and inflammation [3]. Most treatment strategies are based on the cholinergic hypothesis, which postulates that the memory impairment in patients suffering from this disease results from deficient cholinergic function in the brain [4]. One of the most promising approaches for treating this disease is to enhance acetylcholine (ACh) levels in the brain using cholinesterase inhibitors, which block the functions of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), key enzymes that hydrolyze ACh. Increased concentrations of ACh in the brain lead to increased communication between nerve cells and may temporarily improve or stabilize the symptoms of AD [5]. Currently, most drugs used for the treatment of AD are AChE inhibitors such as donepezil, galanthamine and revastigmine, which all have been shown to slow neurodegeneration in AD patients [1]. The limitations of these drugs are their side effects, including aggression, depression, and gastrointestinal disturbances [6]. Furthermore, these drugs are expensive and require weekly blood monitoring [7] ; their effects also "wear off" after a certain period of time [5]. During the search for new treatments for AD, many researchers have focused on spices and herbs, which have a traditional history of use with respect to their cultural heritage and role in maintaining health [8]. Spices, as a category of common plant-based food additives, may provide more than just flavor but may also lower the risk of neurodegenerative diseases, including AD [9]. In this article, we screened 30 commonly used Jordanian spices for their ability to inhibit the activities of AChE and BuChE in AD.

Methods

Plant materials

The 30 spices investigated in this work were purchased from a local Jordanian market in Amman August, 2017. All plant samples were authenticated and voucher specimens were deposited in the herbarium for future reference. The common traditional names, scientific names, and plant parts of the spices examined are summarized in Table 1.

Extraction

Plant materials were cut into small pieces and dried in a hot air oven at 55 °C. Ten grams of pulverized, dried sample were soaked in 100 ml distilled water or 100 ml methanol for 72 h before extraction. Thereafter, the sample was filtered through Whatman filter paper (No. 1). The filtrate was concentrated under reduced pressure at 80 °C using a rotary evaporator and refrigerated at 4 °C until further use.

Chemicals

Acetylthiocholine iodide(ATCI), butyrylthiocholine chloride (BTCI), electric eel AChE (EC 3.1.1.7), horse serum BuChE (EC3.1.1.8), bovine serum albumin, 5,5-dithiobis[2-nitrobenzoic acid] (DTNB), 1-naphtyl acetate, 3,3'-dimethoxybiphenyl-4,4'-di(diazonium) zinc chloride (Fast Blue B salt) and physostigmine were obtained from Sigma (St. Louis, MO). All organic solvents (analytical reagent grade) were purchased from Merck (Darmstadt, Germany).

TLC bioassay

A TLC bioautographic assay was performed as described by Marston et al. [10]. 30 spice extracts (10 µL, 1 mg/mL) and physostigmine were each applied to a silica gel 60 plate coated with F254 (10 cm ×10cm) (Merck, Germany) and were developed with mobile phase, chloroform: methanol (80:20) in a pre-saturated chromatographic chamber. After drying, the TLC plate was sprayed with 13 U/mL AChE dissolved in Tris buffer, pH 7.8 and kept in a water bath at 37 °C for 20 min. The plate was then sprayed with 0.25% 1-naphtyl acetate (dissolved in methanol) and 0.25% Fast Blue B salt (dissolved in deionized water). After a few minutes, a purple background appeared with white spots, indicating the locations of the AChE- and BuChE-inhibiting compounds.

Microplate assay for AChE and BuChE inhibitory activity

All extracts were tested for their ability to inhibit AChE and/or BuChE at a concentration of 1.0 mg/ml using the modified spectrophotometric method developed by Ellman [11]. Positive (physostigmine, 100 µM) and negative (no inhibitor, water) solvent controls were tested. Briefly, 125 µL of 3 mM DTNB, 25 µL of 15 mM ATCI, 50 µL of (Tris/HCl 50 mM, pH 8) buffer, and 25 µL sample dissolved in (Tris/HCl 50 mM, pH 8) buffer containing no more than 10% methanol were added to the wells, followed by the addition of 25 µL of 0.28 U/mL AChE. The absorbance of the reaction mixture was then measured three times at 405 nm every 45 s using a microplate reader (BioTek ELx800, USA). After the third reading, 25 µL of 0.2 U/mL AChE or BuChE in (Tris/HCl 50 mM, pH 8) buffer were added. The final concentration of the plant extract in the first well was 1.0 mg/mL. The absorbance was measured again every 45 s five additional times. The increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the rate of reaction before adding the enzyme from the rate after adding the enzyme. The percent inhibition was calculated by comparing the reaction rates for the sample to the negative control. Results are presented as the mean ± standard errors of the mean (SEM) of each experiment performed in triplicate. For the BuChE assay, the same procedure was followed except that AChE was replaced by BuChE, and ATCI was replaced by BTCI.

Results

30 spices were selected for investigation, and a total of 60 extracts were tested qualitatively for AChE and BuChE inhibition by TLC bioautographic assay followed by the modified spectrophotometric method developed by Ellman to determine a quantitative percent inhibition for the active extracts. Results from the bioautographic assays are expressed as the retention factor (Rf) of the zones of inhibition for AChE and BuChE compared with physostigmine. Eleven of the 30 spice extracts were able to inhibit the enzymatic activity of either AChE or BuChE, or both, as seen in Table 2. The most bioactive extracts against both enzymes occurred with the following spices: white and black pepper, lemon grass, lemon verbena, sumac, turmeric, cumin, fennel, dill, cinnamon and cardamom. The methanolic extracts of these spices were more active, whereas aqueous extract activity was detected in only cumin, dill and fennel. Representative assay results are shown in (figure 1 &2).

The methanolic extracts (spice numbers 2, 4, 6, 8, 11, 12, 13, 16, 17, 25, and 28) had inhibitory Rf values of 0.12, 0.17, 0.20, 0.14, 0.91, 0.88, 0.80, 0.28, 0.45, 0.17, and 0.51 for AChE and Rf values of 0.11, 0.15, 0.22, 0.15, 0.92, 0.88, 0.81, 0.30, 0.45, 0.18, and 0.56 for BuChE, respectively. For the aqueous extracts, spice extracts 11, 12, and 13 were identified as potential inhibitors of AChE (Rf values of 0.12, 0.25, and 0.17, respectively) and BuChE (Rf values of 0.13, 0.27, and 0.15, respectively) compared to physostigmine (Rf values of 0.57 and 0.58 for AChE and BuChE, respectively). The inhibitory activities of the spices examined for AChE were evaluated quantitatively and the percent inhibition of each is shown in Table 2. Experiments were performed in triplicate and are represented as the means ± SEM. Among the 11 active spice extracts, 2 showed potent AChE inhibition, 5 showed moderate inhibition and 4 had low activity. For example, at a concentration of 100 µg/ml, the most potent inhibitory effect (> 90% inhibition) was detected with turmeric and cumin, with percent inhibitions of 93.43 ± 0.64% and 92.14 ± 1.01, respectively. Fennel, dill, lemon grass, lemon verbena and sumac demonstrated moderate activity among the samples tested, with percent inhibitions between 70–80%. In addition, white and black pepper, cinnamon and cardamom had low activity,with percentage inhibitions ranging between 50–60%. The other tested spice extracts did not exhibit any significant AChE or BuChE inhibitory activity. The aqueous extracts from cumin, dill and fennel inhibited both enzymes, with percent inhibitions of 93.16 ± 0.07 and 82.11 ± 0.05; 85.06 ± 0.04 and 77.04 ± 0.22; and 87.1 ± 0.21 and 67.1 ± 0.41 for AChE and BuChE, respectively. To compare the inhibitory activities of the extracts, physostigmine was used as a positive control, because of its well-recognized anticholinesterase activity. Physostigmine inhibited 100% of AChE activity and 85% of BuChE activity at a concentration of 100 µg/mL.

Discussion

The inhibition of AChE and BuChE has been broadly established as a first-line treatment for symptoms of neurodegenerative conditions, such as AD[12]. Three AChE inhibitors have been approved by the U.S. Food and Drug Administration for the treatment of AD: donepezil, rivastigmine and galantamine. However, these drugs are known to have limitations for clinical use due to their short half-lives and/or unfavorable side effects [13]. The development of interventions that substantially delay the onset or progression of AD is currently ongoing. Therefore, the search for new AChE inhibitors is of great interest.

The potential use of natural products has been successfully demonstrated in the field of AD [14]. Natural products(secondarymetabolites) havebeen the most successful source of potential drug leads for new bioactive AChE inhibitors which, according to the cholinergic hypothesis, increases the levels of ACh in the brain, improves cholinergic function in patients with AD and alleviates symptoms of this neurological disorder [15].

A great deal of attention has been focused on herbs and spices as sources of drugs. While the scientific evidence for the use of common herbs and spices is lacking, the beneficial effects observed from their use are generally encouraging [8]. There is a need for a more comprehensive understanding of the health-promoting and protective properties of herbs and spices. A spice is a dried seed, fruit, root, bark or flower of a plant used in small quantities in food for flavor, color, or as a preservative. Spices have been evaluated by researchers for the treatment of many diseases far removed from their traditional uses [16]. The present study was undertaken to evaluate the anticholinesterase activity of a number of common Jordanian spices to provide science-based evidence for their use as preventive and therapeutic agents for AD.

We investigated 30 spices from 17 families for their ability to inhibit AChE and BuChE using a TLC bioautographic assay and Ellman's spectrophotometric method. Among the 30 screened extracts, 11 had AChE and/or BuChE. The anti-cholinesterase activities of these spices are summarized in Table 3. However, most of the cited studies focused on the inhibition of AChE but not BuChE. BuChE plays an equally important role to that of AChE, as the sole inhibition of AChE leads to a compensatory mechanism by which the activity of BuChE is increased. Five of the active spice extracts were screened for their ability to inhibit BuChE, supporting their roles as preventive and therapeutic agents for AD with dual inhibitory activities. To the best of our knowledge, we report for the first time that fennel seed extract is able to inhibit both AChE and BuChE.

The results of this study indicate that the consumption of spices and phytochemicals can greatly impact the onset and progression of chronic neurodegenerative diseases, such as AD. Public health efforts should be directed to increase the consumption of these spices as part of a regular diet that includes protective phytonutrients to help prevent or alleviate symptoms in patients suffering from AD. Among the most common Jordanian spices, white and black pepper, lemon grass, lemon verbena, sumac, turmeric, cumin, fennel, dill, cinnamon and cardamom have the greatest potential for lowering the risk of neurodegenerative diseases, including AD. These spices should be considered candidate treatments for AD. Further investigations (isolation, purification, and structural determination of the active constituents of these spices via bioassay-directed fractionation) are needed) to confirm their potential to treat AD.

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Table 1. Commonly used Jordanian spices selected for screening

No.	Common Names	Scientific Name	Family	Part Used
1	Anise	**Pimpinella anisum L.**	Apiaceae	Seeds
2	White pepper	**Piper nigrum L.**	Piperaceae	Ripe fruit seeds
3	Bay laurel	**Laurus nobilis L.**	Lauraceae	Leaves
4	Black pepper	**Piper nigrum L.**	Piperaceae	Unripe fruit seeds
5	Capsicum	**Capsicum annuum L.**	Solanaceae	Fruits
6	Cardamom	**Elettaria cardamomum L.**	Zingiberaceae	Seeds
7	Carthamus	**Carthamus lanatus L.**	Asteraceae	Flowers
8	Cinnamon	**Cinnamomum zeylanicum Nees**	Lauraceae	Bark
9	Clove	**Syzygium aromaticum L. Merrill & Perry**	Myrtaceae	Flower buds
10	Coriander	**Coriandrum sativum L.**	Apiaceae	Seeds
11	Cumin	**Cuminum cyminum L.**	Apiaceae	Seeds
12	Dill	**Anethum graveolens L.**	Apiaceae	Seeds
13	Fennel	**Foeniculum vulgare Mill.**	Apiaceae	Seeds
14	Fenugreek	**Trigonella foenum-graecum L.**	Fabaceae	Seeds
15	Ginger	**Zingiber officinale Roscoe**	Zingiberaceae	Rhizomes
16	Lemon grass	**Cymbopogon schoenanthus L.**	Poaceae	Stems and leaves
17	Lemon verbena	**Aloysia triphylla (L'Hér.) Britton**	Verbenaceae	Leaves
18	Nutmeg	**Myristica fragrans Houtt.**	Myristicaceae	Seeds
19	Nutmeg flower	**Nigella sativa L.**	Ranunculaceae	Seeds
20	Parsley	**Petroselinum crispum Mill. Fuss**	Apiaceae	Leaves
21	Peppermint	**Mentha spicata L.**	Lamiaceae	Leaves
22	Rosemary	**Rosmarinus officinalis L.**	Lamiaceae	Leaves
23	Saffron	**Crocus sativus L.**	Iridaceae	Flowers
24	Sage	**Salvia fruticosa Mill.**	Lamiaceae	Flowers
25	Sumac	**Rhus coriaria L.**	Anacardiaceae	Flowers
26	Thyme	**Thymus vulgaris L.**	Lamiaceae	Leaves
27	Thyme	**Thymus capitatus L. Hoffmanns. & Link**	Lamiaceae	Leaves
28	Turmeric	**Curcuma longa L.**	Zingiberaceae	Rhizomes
29	Verthemia	**Chiliadenus iphionoides (Boiss. & Blanche) Brullo**	Asteraceae	Flowers
30	Basswood	**Tilia cordata Mill.**	Malvaceae	Leaves

Table 2: Inhibition of AChE and BuChE by plant extracts

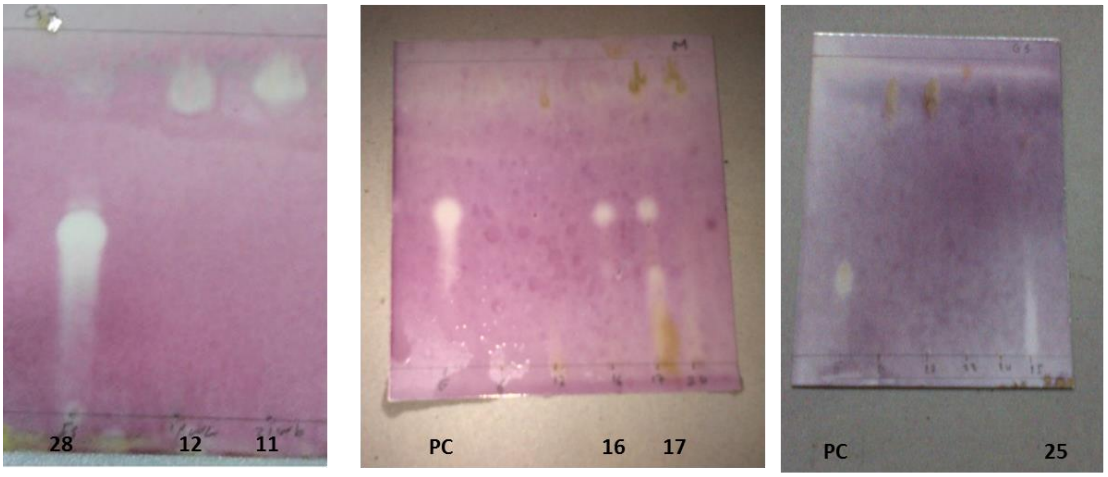
No.	Common Name	Inhibition (%) AChE ± SEM		Inhibition (%) BuChE ± SEM	
		Methanolic (100 µg/ml)	Aqueous (100 µg/ml)	Methanolic (100 µg/ml)	Aqueous (100 µg/ml)
2	White pepper	64.07 ± 0.77	NA	54.07 ± 0.45	NA
4	Black pepper	60.02 ± 0.31	NA	56.02 ± 0.21	NA
6	Cardamom	54.98 ± 1.07	NA	50.03 ± 0.78	NA
8	Cinnamon	61.32 ± 0.58	NA	46.04 ± 0.22	NA
11	Cumin	92.14 ± 1.01	93.16 ± 0.07	80 ± 0.04	82.11 ± 0.05
12	Dill	77.02 ± 0.31	85.06 ± 0.04	78.1 ± 002	77.04 ± 0.22
13	Fennel	80.05 ± 0.02	87.1 ± 0.21	66.2 ± 0.3	67.1 ± 0.41
16	Lemon grass	75.99 ± 0.07	NA	80.11 ± 0.01	NA
17	Lemon verbena	78.03 ± 0.065	NA	68.03 ± 0.08	NA
25	Sumac	75.05 ± 1.2	NA	65.5 ± 0.05	NA
28	Turmeric	93.43 ± 0.64	NA	84.5 ± 0.56	NA

NA: No activity; SEM: Standard Error of the Mean

Table 3: Reported AChE and BuChE inhibition of 11 active spices

No.	Spice Name	Reported Inhibition of AChE	BuChE	Reference
1	Turmeric	√	-	[17-20]
2	Cumin	√	√	[21-23]
3	Fennel	-	-	-
4	Lemon grass	√	-	[27-30]
5	Lemon verbena	√	-	[23]
6	Dill	√	-	[24-25]
7	Sumac	√	-	[26]
8	Cardamom	√	√	[7, 22]
9	Cinnamon	√	√	[7,22]
10	White pepper	√	√	[7]
11	Black pepper	√	√	[7]

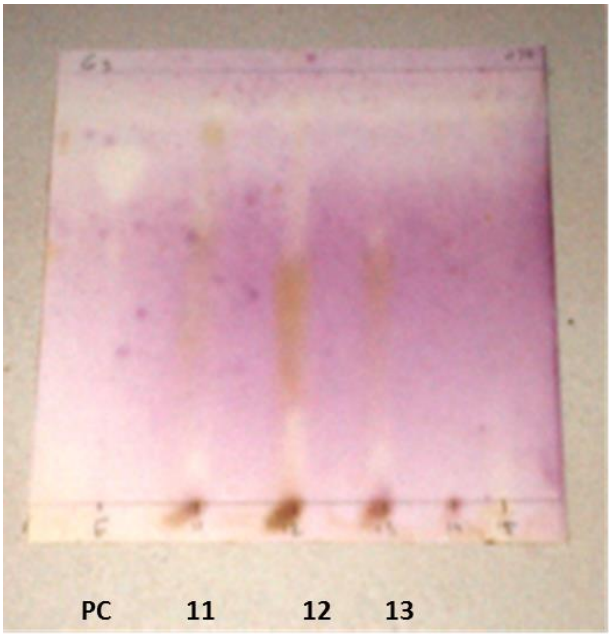
Figure 1. TLC assay results for AChE and BuChE inhibition by methanolic extracts of spices 11, 12, 16, 17, 25 and 28



A B C

PC: positive control, physostigmine, A & B: AChE inhibition; C: BuChE inhibition

Figure 2. TLC assay results for AChE inhibition by aqueous extracts of spices 11, 12, and 13. PC: positive control, physostigmine.



PC: positive control, physostigmine.